# Influence of menthol on human temperature regulation and perception

By

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# Abstract

When exercise is undertaken in warm, humid conditions, the thermal gradient between the skin and environment, and the capacity for evaporative heat loss, are reduced. These factors, along with an increase in metabolic heat production, lower work capacity and exercise performance. Thermoreceptors located within the skin and deep in the body convey information on this accumulation of thermal energy to higher brain structures and, if mean body temperature rises uncontrollably, the cumulative neuronal input is thought to produce inhibitory signals that lower work capacity, such that metabolic heat production decreases to protect the organism from heat injury. Lessening these inhibitory signals may enhance or help to maintain exercise performance in the heat. The inhibitory signals might be lessened by cooling the skin and deep body temperature prior to or during exercise, or perhaps by applying menthol on the skin, or some combination of these.

Menthol is a chemical compound that activates cold receptors (TRPM8) in the skin to elicit cool sensations. These receptors are not otherwise activated unless cooled below 27 °C. Hence, menthol, when applied to the skin of heat stressed humans, may provide a "cool'' neuronal input to higher brain structures in addition to the neuronal signals arising from warm thermoreceptors located within the body. But menthol may also induce a heat storage (cold defense) response that would then heighten the activity of warm receptors deep in the body. Therefore, it is not clear whether menthol might reduce, enhance or help to maintain exercise performance in heat stressed humans. Moreover, no studies have assessed the perceptual and thermoregulatory response to menthol during rest or exercise, or the consequence of its repeated use. Before it is recommended as a possible ergogenic aid, these studies should be undertaken. The early work presented in this thesis tested the hypotheses that a water-based spray, containing ethanol and/or menthol, would enhance evaporative cooling when sprayed on the skin, thereby lowering heat storage and improving thermal perception compared to an unsprayed Control condition; but menthol would also improve thermal perception independent of temperature by directly stimulating cold receptors, during rest and exercise in warm, humid conditions. The hypothesis that menthol-mediated cool sensations would not undergo any habituation after repeated exposures was also tested.

The general approach to testing these hypotheses involved presenting human participants with a thermal challenge that would induce warm sensations and increase thermal discomfort, whilst encouraging a level of heat storage that could be compensated for by increasing heat loss through vasodilation and sweating. This was achieved by manipulating metabolic heat production through a combination of rest and fixed intensity exercise in warm (30 °C) and humid (70 %) conditions. The influence of a menthol solution spray was tested against the backdrop of this thermal challenge.

The results supported the general hypothesis that a water-based upper-body spray containing menthol can increase sensations of coolth compared to no spraying or water-only spraying during rest and exercise in warm, humid conditions, but menthol also influences body temperature regulation. The effect that menthol exerts over perception and thermoregulation differs by dose and fades with time. Specifically, 0.2 % menthol spraying encourages heat storage by enhancing vasoconstriction, and there is no habituation in these responses. 0.05 % menthol spraying did not encourage any additional heat storage compared to a Control spray. Menthol also influenced perception, with a 0.2 % menthol spray promoting cooler sensations and greater irritation than 0.05 % menthol and Control spraying. Compared to a Control spray, 0.2 % menthol reduced thermal comfort during rest and improved it during exercise, while 0.05 % menthol did not alter thermal comfort during rest, and may have improved it during exercise. Neither menthol spray influenced perceived exertion during exercise. Menthol-mediated cool sensations lasted 15 to 30 minutes. Both 0.2 % and 0.05 % menthol sprays underwent an habituation compared to the Control spray, with cool sensations diminishing after repeated daily exposures.

It is concluded that a 0.05 % menthol spray, which induces cool sensations *without* a significant heat storage response, could be considered as a perceptual cooling intervention with some capacity to enhance evaporative heat loss when sprayed on the skin during rest and moderate fixed-intensity exercise in the heat. A 0.2 % menthol spray might be deployed later in exercise, but may increase heat storage and irritation. Further testing is required to identify whether menthol spraying improves maximal exercise performance.

## Declaration

Whilst registered as a candidate for the degree of doctor of philosophy, I have not been registered for any other research award. The results and conclusions embodied in this thesis are the work of the named candidate and have not been submitted for any other academic award.

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## Glossary of symbols & abbreviations

| | |
|---|---|
| **° C** | Degrees Celsius |
| Δ | Change |
| **ANOVA** | Analysis of variance |
| **BSA** | Body surface area |
| ***C*** | Convective thermal exchanges |
| **$CO_2$** | Carbon dioxide |
| **CON** | Control spray |
| ***E*** | Evaporative thermal exchanges |
| **fMRI** | Functional magnetic resonance imaging |
| **$F_{ECO_2}$** | Fraction of expired carbon dioxide |
| **$F_{EO_2}$** | Fraction of expired oxygen |
| **HR** | Heart rate |
| **IRR** | Irritation |
| **J** | joule |
| ***K*** | Conductive thermal exchanges |
| **kJ** | Kilojoule |
| **KPa** | Kilopascal |
| **LMS** | Labeled magnitude scale |
| **$M_{0.05\%}$** | 0.05 % menthol spray |
| **$M_{0.2\%}$** | 0.2 % menthol spray |
| ***M*** | Metabolic energy transformation |
| **M/E** | 0.2 % menthol + 20 % ethanol |
| **nL** | nanolitres |
| **$O_2$** | Oxygen |
| **PO** | Power output |
| ***R*** | Radiative thermal exchanges |
| **RCI** | Reciprocal cross inhibition |
| **RER** | Respiratory exchange ratio |
| **rh** | Relative humidity |
| **RPE** | Rating of perceived exertion |
| **rpm** | Revolutions per minute |
| ***S*** | Stored thermal energy |
| **SD** | Standard deviation |
| **SkBF** | Skin blood flow |
| **SR** | Sweat rate |
| **$\bar{T}_b$** | Mean body temperature |
| **TC** | Thermal comfort |
| **$\bar{T}_{msk}$** | Mean skin temperature |
| **TRP** | Transient receptor potential |
| **$T_{re}$** | Rectal temperature |
| **TS** | Thermal sensation |
| **$\dot{V}_E$** | Minute ventilation |
| **$\dot{V}O_2$** | Rate of oxygen consumption |
| **$\dot{V}CO_2$** | Rate of carbon dioxide production |
| **W** | watt |
| ***W*** | Mechanical work |
| **WA** | Water only spray condition |

# Chapter 1

# Introduction

When exercise is undertaken in warm, humid conditions, the thermal gradient between the skin and environment, and the capacity for evaporative heat loss, are reduced. These factors, along with an increase in metabolic heat production, contribute to an accumulation of body heat that is associated with reductions in work capacity (Rowell *et al.,* 1966).

This fatigue, which is associated with dysfunction in multiple systems, is probably due to an interaction between elevations in mean body temperature and cardiovascular strain, rather than an absolute critical level of core temperature alone (Gonzalez-Alonso *et al.,* 2008); but central brain structures have also been implicated. During fixed-intensity exercise in the heat, Nybo and Nielsen (2001) showed that fatigue is associated with an increase in perceived exertion and a gradual slowing of brain activity (electroencephalography), with no associated reduction in the activation pattern of muscles (electromyography). This suggests that central brain structures may also exert an influence on fatigue in addition to the muscles.

A number of studies using ratings of perceived exertion (RPE) as an indicator of higher brain function have sought to clarify the factors that drive this 'central' fatigue in the heat. Interestingly, although Nybo and Nielsen (2001) showed that RPE is highly associated with increases in deep body temperature, this is not always so; RPE is also influenced by other factors, like skin temperature. For example, Tucker *et al.,* (2006) observed a reduction in the self-selected power output of individuals in the first few minutes of exercise undertaken in hot, compared to cool conditions, and this reduction occurred before deep body temperature differed between conditions. The altered pace was associated with an increase in skin temperature, suggesting that peripheral thermoreceptors sensed the higher ambient temperature in the hot condition and influenced the reduction in pace (Tucker *et al.,* 2006). In support of this notion, Schlader *et al.,* (2011b) showed that skin temperature, and the associated perceptions of comfort and sensation, are all important inputs in the initial selection of pace. Taken together, these findings suggest that when exercise is undertaken in hot, compared to cool conditions, thermoreceptors located in the skin and deeper tissues of the body convey information about the accumulation of thermal energy to higher structures in the brain. It is thought that the cumulative neuronal input

from these thermoreceptors gives rise to inhibitory signals that lower power output (lowering metabolic heat production) to protect the organism from heat injury (Nybo, 2010). It is not clear how or where this higher processing occurs, but it probably arises from interactions of brain areas in large scale distributed networks (Menon & Uddin, 2010) that include structures for perception (thalamus, cerebral cortex; Craig, 2002) and thermoregulation (hypothalamus; Morrison & Nakamura, 2011; Romanovsky, 2007).

Lessening the inhibitory signals during exercise in the heat may enhance or help to maintain exercise performance. Given the inhibitory signals seem to be partly mediated by warm thermoreceptor activation; they might be attenuated by cooling the skin and/or deep body prior to or during exercise in the heat. This may improve performance by lowering the activity in warm receptors, and increasing the activity in cold receptors. There is a broad literature assessing the effectiveness of various cooling interventions (ice vests, water immersion), many of which are impractical in an actual sporting or working scenario (Cheung, 2010a; Duffield, 2008). A simple cooling strategy that is easily implemented in a variety of scenarios involves wetting the skin by water spraying. This method can enhance evaporative heat loss from the skin, thereby lowering skin temperature during exercise in warm, humid conditions (Bassett *et al.,* 1987), but may also increase cool sensations and reduce perceptions of heat stress, which may enhance work intensity (Cheung, 2010b; Schlader *et al.,* 2011a). For these reasons, the effectiveness of a cooling strategy should be measured not only by the reduction in mean body temperature it provides, but also by the improvement it affords perception.

In an effort to enhance evaporative heat loss and improve cool sensations in the heat, commercial companies have added menthol and ethanol to their water-based skin cooling products (Physicool™, London, U.K; Energizer™ Liquid Ice CosMedicals Inc. AG, Switzerland; ICE, Skins™, NSW, Australia). Menthol is a chemical compound that activates cold receptors (TRPM8) in the skin to elicit cool sensations in humans (Hensel, 1981). As an important side note, the TRPM8 receptor does not otherwise activate unless a temperature lower than 27 °C is encountered (McKemy *et al.,* 2002; Peier *et al.,* 2002). Hence, menthol, when applied to the skin of heat stressed humans, provides a cool neuronal input that is equivalent to encountering a temperature of 27 °C or cooler, in addition to the neuronal signals arising from warm thermoreceptors within the body. But menthol may also induce a heat storage response (Kounalakis *et al.,* 2010), which may

accentuate the neuronal output of warm receptors deep in the body. Ethanol, on the other hand is an alcohol that vaporises more quickly than water or sweat, and has the potential to increase evaporative heat loss (Godts *et al.,* 2005), thereby providing a cool input to higher brain structures.

The benefit of using an ethanol and/or menthol-based water spray over a water-only spray, or no spraying, is not clear. To date, only one other study has assessed the use of a combined ethanol/menthol solution on performance. Very recently, Mujika *et al.,* (2010) provided highly trained rowers with forearm sweatbands soaked in either a cooling solution containing ethanol, menthol and water (Energizer™ Liquid Ice CosMedicals Inc. AG, Switzerland;) or water alone (NB. no Control condition), during an indoor 2000 m self-paced time trial. The authors observed no significant difference in perceived exertion, time to finish, or pacing strategy between the interventions. However, the evaporative cooling capacity of this intervention was limited because the surface area exposed to the solution was small (forearms only), also because the sweat bands created an additional barrier to evaporative heat loss between the skin and the environment. Lastly, the possible negative influence of the ethanol/menthol solution on thermoregulation could not be assessed because of the self-paced study design, which did not control for metabolic heat production. Further research is required to clarify the influence of a menthol/ethanol solution on thermoregulation and perception during fixed-rate exercise, when metabolic heat production is standardised.

***General research questions and hypotheses***

The studies reported in this thesis examined whether spraying an ethanol and/or menthol solution on the skin during rest and fixed-intensity exercise in warm, humid conditions improved evaporative cooling or sensations of coolth. The impact of repeated spraying was also studied. It was hypothesised that spraying the skin with menthol, combined with either ethanol or water, would enhance evaporative cooling compared to no spraying, and that menthol would improve sensations of coolth more than sweating or water spraying alone. It was further hypothesised that there would be no habituation of the initial responses evoked by menthol after repeated exposure.

***Overview of the thesis***

In chapter two, the influence of menthol on temperature perception and regulation in warm, humid conditions is reviewed. In chapter three, the methodological and technical

issues related to each research question are described. The experimental studies undertaken to clarify the influence of menthol on perception and thermoregulation are presented in chapters four to seven; their findings are summarised below.

Before outlining the results of Study one, the rationale for undertaking it should be mentioned. In the run-up to the Beijing Olympics of 2008, UK Sport requested the Extreme Environments Laboratory at Portsmouth University to test a commercially available cooling solution spray (Physicool™, London, U.K; Energizer™) composed of 0.2 % menthol and 20 % ethanol (in 80 mL of water). UK Sport questioned whether the combined menthol and ethanol spray could alleviate heat stress amongst athletes and support staff working in Beijing, the latter might spend hours resting or moderately walking in the warm, humid climate (30 °C, 70 % rh). For this reason, Study one (Chapter four) compared a 0.2 % menthol + 20 % ethanol-based water spray to a water-only spray and a Control group (un-sprayed) during light stepping exercise in these conditions. The results suggested that ethanol/menthol spraying improved evaporative cooling compared to water spraying and no spraying in the short term, but it was comparable to water spraying beyond 30 minutes. Both sprays enhanced evaporation compared to no spraying. Menthol/ ethanol/water spraying increased heat storage compared to water spraying, but also caused the coolest sensations compared to all other conditions; but it neither improved, nor reduced thermal comfort, possibly due to an interaction with irritation. Given that water spraying cooled the skin comparably to the ethanol/menthol/water spray beyond 30 minutes, without inducing any heat storage response, and was more cost effective, it was recommended to UK Sport over the menthol/ethanol spray. But the perceptual cooling power of the menthol/ethanol spray was intriguing and hypothesised to be due to the action of menthol; raising the possibility of using it as a perceptual cooling aid in hot conditions, but little was known of menthol's influence on human perception and thermoregulation during rest or exercise.

Study two (Chapter five) sought to characterise the influence of 0.2 % menthol on heat storage and thermal perceptions, in the absence of ethanol, with the aim of assessing its viability as a perceptual cooling aid. A 0.2 % menthol/water spray was compared to a water-only spray (Control) during rest and exercise in warm, humid conditions. The 0.2 % menthol spray induced slight heat storage, but the practical consequence of this response and the underlying mechanisms driving it were not clear. Menthol spraying also resulted in cooler sensations and reduced thermal comfort at rest, but not during exercise, possibly due

to an interaction with irritation. These responses were attributed to menthol-mediated activation of the TRPM8 cold receptor. It has been suggested that cool sensation varies directly with menthol dose, as does irritation, but cool sensations may vary less than irritation. Hence, lowering the 0.2 % menthol dose may reduce the negative sensations of irritation and preserve cool sensations, thereby optimising the perceptual influence of the cooling spray, but this required clarification.

Study three (Chapter six) explored whether lowering the dose of menthol from 0.2 % to 0.05 % could minimize irritation and improve thermal comfort whilst maintaining cool sensations in warm, humid conditions. The second aim was to characterise the underlying mechanisms driving heat storage following application of the 0.2 % menthol spray. Results indicated that the heat storage following 0.2 % menthol spraying was induced by vasoconstriction at rest, rather than by a withdrawal of sudomotor function during exercise. 0.05 % menthol spraying did not increase heat storage compared to the Control spray. Lowering the dose of menthol from 0.2 % to 0.05 % also preserved sensations of coolth, reduced irritation, and did not influence thermal comfort. These findings raised the possibility of using a 0.05 % menthol spray as a perceptual cooling aid, with some capacity to enhance evaporative heat loss depending upon sweat production and environmental humidity, during exercise in the heat. In a realistic setting, such a cooling aid could be used repeatedly, perhaps daily, raising the question as to whether users may habituate to menthol and lose the perceptual benefit.

Study four (Chapter seven) examined whether the menthol-mediated sensation of coolth habituated after repeated exposure to a high (0.2 %) or low (0.05 %) dose menthol solution spray, and whether repeated 0.2 % menthol spraying caused a reduction in heat storage in either warm or cool conditions. Results indicated that there was no habituation of the heat storage response after repeated exposure to 0.2 % menthol, and this response was mediated by an increase in vasoconstrictor tone. In contrast, thermal sensation underwent an habituation, most significantly after 0.2 % menthol spraying. These findings raise the possibility of using a lower dose (0.05 %) menthol solution spray to enhance evaporative cooling and sensations of coolth during moderate fixed-intensity exercise in the heat.

The last four chapters of the thesis include a general discussion (Chapter 8), conclusions and recommendations (Chapter 9), assumptions, limitations, and delimitations (Chapter 10) and recommendations for future experiments (Chapter 11).

# Chapter 2

# Review of the literature

This literature review explores menthol's influence on human temperature regulation and perception. It begins by considering the biophysics of heat exchange in warm, humid conditions and then describes how menthol is initially detected. The afferent pathways and higher brain structures through which it is thought to exert its influence are then described. Following this, menthol's influence on body temperature regulation is considered, along with its influence on vasomotor and sudomotor function. The perceptual consequences of menthol exposure are then considered; specifically, its influence on temperature sensation, irritation, thermal comfort and perceived exertion. The review concludes by considering whether there is any likely perceptual or physiological habituation to repeated menthol use.

The peer reviewed publications included in this narrative literature review were retrieved from a comprehensive literature search of electronic databases and cited references. The electronic search primarily included MEDLINE/PubMed; however all of the references that were retrieved were also scanned for relevant citations to expand the search.

## The biophysics of heat transfer in warm, humid conditions

This section will review key biophysical principles that govern heat transfer in warm, humid conditions (30 °C, 70 % rh). This level of heat stress was chosen to simulate the warmth and humidity that could be encountered during the Beijing Olympics of 2008. As previously noted, Study one of this thesis was undertaken in partnership with UK Sport, who asked whether a menthol and ethanol-based cooling spray could alleviate heat strain amongst athletes and support staff attending the 2008 Olympic Games in Beijing. The results from this initial study suggested that the warm, humid conditions presented an adequate environment to assess the effectiveness of a menthol-based cooling intervention because it imposed a thermal challenge that induced warm sensations and increased thermal discomfort in human participants, but also encouraged a level of body heat storage that could be compensated for by enhancing vasodilation and sweating; hence, menthol's influence on body temperature regulation could be assessed.

The thermal challenge imposed by any environment can be described by the laws of thermodynamics, which dictate the magnitude, direction and rate of thermal exchange

between a body and its environment. The heat balance equation allows thermal physiologists to apply the first law of thermodynamics *i.e.* conservation of energy, to quantify these thermal exchanges. This equation, presented below (Equation 1), shows how the amount of stored thermal energy (*S*) in a body results from the balance among evaporative (*E*), radiative (*R*), convective (*C*) and conductive *(K*) thermal exchanges, and heat produced through metabolic energy transformation (*M*) and exchanged when performing mechanical work (*W*) (IUPS Thermal Commission, 2001). These variables are shown in equation one:

$$\textbf{\textit{Equation 1}} \qquad S = M - (\pm W) - E \pm R \pm K \pm C \ [\mathrm{W \cdot m^{-2}}]$$

The second law of thermodynamics states that heat energy flows down a gradient, dictating the direction of all thermal exchange between an individual and the environment. Hence, deep body, skin and ambient air temperature are all vital determinants of heat exchange. Relative humidity also influences heat dissipation and it is influenced by the amount of water vapour in the air, which has a positive relationship with the vapour pressure in the air. Vaporised water molecules move toward equilibrium, travelling down a gradient from high to low pressure. This molecular motion dictates that evaporated water is more readily accepted in a low, as opposed to a highly humid environment. Evaporative heat loss occurs when sweat, water, or another liquid (like ethanol) stores up sufficient thermal energy from the skin to undergo a phase change from liquid to gas; during the change heat is removed, and the skin is cooled. But, if the vapour pressure in the air is equal to or higher than that measured at the skin, the pressure gradient can be reduced or reversed, and sweat will not completely vaporise; thereby, reducing evaporative heat loss to the environment. In the work presented in this thesis, relative humidity remained constant around 70 % (30 °C dry bulb temperature, 26 °C wet bulb temperature), which is equivalent to a water vapour pressure of 2.97 Kilopascals (Kpa). In order to optimise evaporative heat loss in this environment, the vapour pressure at the skin surface must rise above 2.97 Kpa. To promote this, an individual can increase sweat production, or saturate the skin surface with a liquid such as water or ethanol. This oversaturation, combined with an elevation in mean skin temperature commonly encountered during exercise in the heat (*i.e.* 35 °C), can result in an estimated vapour pressure at the skin of 5.9 Kpa. With this favourable pressure gradient, liquid can freely evaporate from the skin.

The amount of thermal energy a given liquid can draw from the skin as it evaporates is referred to as its latent heat of vaporisation; this differs between liquids. For example, the

amount of thermal energy required to evaporate one gram of ethanol is 920 joules, while one gram of water requires 2,450 joules (Godts *et al.,* 2005). One gram of ethanol will evaporate five times faster than one gram of water, but its absolute capacity to remove heat from the skin is nearly one third that of water (Godts *et al.,* 2005). This suggests that if ethanol is repeatedly applied on the skin, it has the potential to enhance evaporative heat loss compared to water, due to its faster rate of vaporisation. This was the rational for repeatedly spraying participants with the menthol/ethanol spray in Study one.

Radiative heat loss occurs from all objects possessing thermal energy; in humans, thermal radiation leaves the skin surface in the form of wave energy. Radiative heat loss is influenced by numerous factors, including clothing insulation and air ventilation. In the studies undertaken in this thesis any differences in radiative heat losses between conditions were hopefully mitigated, as participants wore the same clothing ensembles and encountered the same environmental conditions with each visit to the laboratory. Conductive heat losses are small and only occur when the skin (31 °C to 33 °C at rest) comes into contact with an object that is cooler than itself; therefore, conductive heat losses are negligible in the studies presented in this thesis. Convective heat losses occur when the medium surrounding the skin is cooler than the skin itself; in this case, thermal energy moves down its thermal gradient and is replaced by cooler air. This process creates thermal currents that end in a net movement of thermal energy away from the skin. The use of a fan, common during laboratory-based exercise, can increase convective heat losses substantially by introducing forced convection.

Under the conditions of the studies undertaken in this thesis, metabolic heat production represented the greatest source of heat gain in the human body.

**Thermoreceptors, afferent pathways to higher brain structures and menthol**

Small diameter myelinated Aδ and un-myelinated C primary afferent fibers innervate all tissues of the body and convey the chemical, mechanical, metabolic and hormonal status of the skin, muscles, joints and viscera to higher brain structures (Craig, 2003). The mechanisms by which thermal stimuli are converted to neural input have only recently been understood. It is now agreed that transient receptor potential (TRP) ion channels embedded in the terminals of these primary afferent nerve endings influence cellular activity in response to thermal energy, and in some cases, chemical compounds. Although as many as six separate families of TRP ion channels have been identified, containing 28

different channels, only three families are thought to act as thermoreceptors; namely, the vanilloid TRP channels (TRPV), the melastatin TRP channel, (TRPM), and the ankyrin transmembrane protein channel (TRPA) (Schepers & Ringkamp, 2008). These receptors, shown below in Figure 1, allow humans to sense a wide range of temperatures, from noxious (painful) cold and heat, to innocuous (non-painful) warmth and coolth.

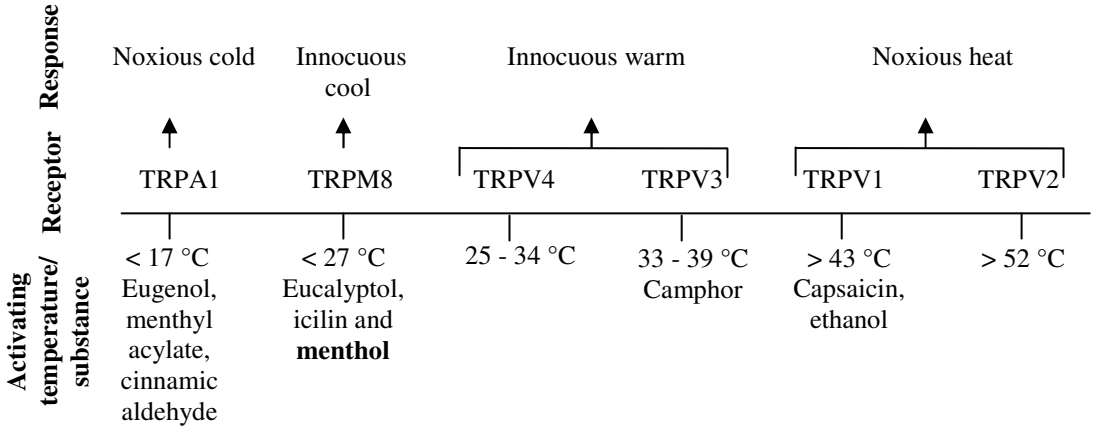


**Figure 1**. Thermoreceptor activation by various temperatures and substances.

Cool temperatures in the innocuous range are sensed by the TRP melastatin-eight (TRPM8) ion channel, which is activated by temperatures below 27 °C, as well as chemical compounds including eucalyptol, icilin (Jordt *et al.,* 2003) and, most pertinent to the work undertaken in this thesis, menthol (McKemy *et al.,* 2002; Peier *et al.,* 2002).

Menthol ($C_{10}H_{20}O$; molecular weight, 156) is a cyclic terpene alcohol produced from mint oils or prepared synthetically (Eccles, 1994). It is found in many active forms; however, the L isomer is most commonly used in commercial products because it produces the strongest cooling effects and is nontoxic to humans (Eccles *et al.,* 1988). It must be noted that up to 50 % of primary neurons which respond to cold temperatures and menthol also have the noxious heat receptor TRPV1 (McKemy *et al.,* 2002); therefore, Green (2004) has suggested that some of the neurons that posses the TRPM8 receptor may project to the nociceptive (pain mediating) pathway rather than, or along with, the cold pathway.

Given sufficient stimulation, such as a sudden change in skin temperature or skin surface application of menthol, primary sensory neurons depolarise and action potentials propagate towards a cell body in the dorsal root ganglion. The signal continues along the central axon towards the cell body of a second order neuron inside the spinal cord at lamina I of the dorsal horn. The axon of this second order neuron then exits the grey matter of the dorsal horn, cross the midline, and ascends contra-laterally in the white matter of the lateral

spinothalamic tract. These ascending neuronal projections, often referred to as 'labelled lines' because they carry specific information, can be divided into three categories, or those that propagate action potentials along the peripheral axon which respond to 1) noxious, mechanical *and* heat stimuli (nociceptive specific neurons), 2) noxious, mechanical, heat *and* cold stimuli (polymodal nociceptive neurons), and 3) those that respond in a linear fashion to innocuous warming or cooling, but are *not* activated by noxious temperatures (thermoreceptive specific neurons) (Craig, 2002). Although it is not entirely clear which 'labelled lines' primarily influence our perception of temperature, Morrison and Nakamura (2011) contend that thermoreceptive specific neurons drive body temperature regulation. The distinction is important because the information carried by the warm and cold sensitive neurons ascending in lamina I will be used in two ways, depending upon where they branch and ultimately synapse. Within lamina I for example, one branch projects toward the thalamus and onto the somatosensory cortex, contributing to temperature perception (Craig, 2002). Another branch carrying the same information synapses on the lateral parabrachial nucleus and is used as an input to regulate body temperature (Morrison & Nakamura, 2011).

## Body temperature regulation and menthol

Thermoreceptors located within the body convey thermal information to higher brain structures; this information is then integrated in the hypothalamus (Romanovsky, 2007). Cold and heat defence responses are driven by two distinct areas in the hypothalamus (Morrison & Nakamura, 2011), but it is not clear how the hypothalamus integrates the information and triggers these responses. One theory suggests that the neural pathways for cold and heat defence communicate with each other whereby activation of one inhibits the other in a process referred to as reciprocal cross inhibition (Sherrington, 1906; Bazett, 1949; Bligh, 1998); but it is also possible that each pathway is independent (Kobayashi *et al.,* 2006). In any case, observations made on resting (Savage & Brengelmann, 1996) and exercising (Franks *et al.,* 1996) humans suggest that the regulated variable in the whole system is an integrated mean body temperature, which is probably derived from the cumulative input from thermoreceptors located within the body (Werner *et al.,* 2008).

### *Body temperature regulation at rest*

During rest, mean body temperature is regulated by altering skin blood flow, such that vasoconstriction lowers the amount of thermal energy that is transferred from the body to the environment, and vasodilation increases it. Savage and Brengelmann (1996) showed

this by spraying water (either 33 °C or 35 °C) on the skin of resting participants and monitoring the change in skin and esophageal temperature, and forearm skin blood flow. The authors showed that the fall in skin temperature from water spraying reduced skin blood flow, which lowered heat loss and raised esophageal temperature. Importantly, the authors observed an inverse relationship between skin and deep body temperature, which indicates that mean body temperature perhaps remained stable, although this calculation was not reported. The study by Savage and Brengelmann (1996) also showed that skin temperature fluctuations in the range of 33 °C to 35 °C could be compensated for by altering vasomotion alone. This range, shown below in Figure 2, has been associated with thermoneutrality in humans (Mekjavic & Eiken, 2006).

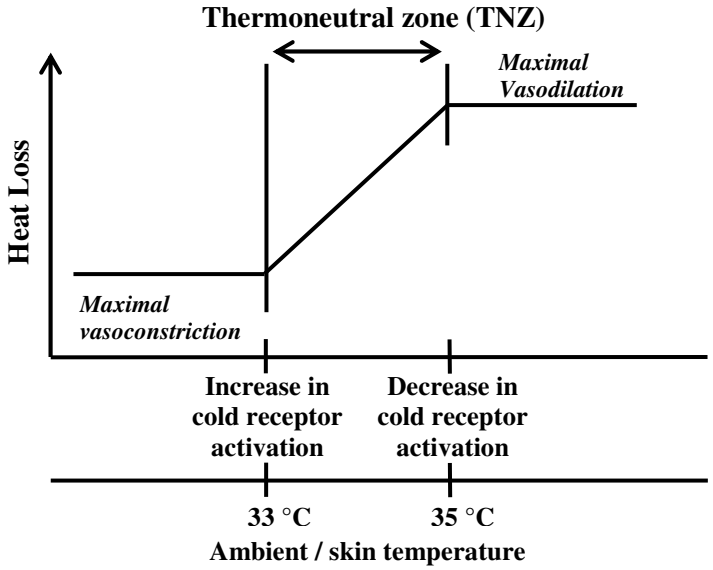


**Figure 2**. Thermoneutral zone (TNZ) in humans (adapted from Mekjavic & Eiken, 2006).

The IUPS Thermal Commission (2001) defines the thermoneutral zone (TNZ) as the range of ambient temperature at which thermoregulation is achieved without changing metabolic heat production or evaporative heat loss. Within a given species, the TNZ will differ depending upon insulation (*i.e.* sub-cutaneous fat), posture, metabolic rate, experimental conditions and ambient temperature (IUPS Thermal Commission, 2001; Romanovsky *et al.,* 2002). This suggests that the skin temperatures associated with maximal vasoconstriction and vasodilation that border the TNZ in Figure 2 can change, so labelling the horizontal axis with the skin temperatures associated with thermoneutrality (*i.e.* 33 °C and 35 °C, Savage & Brengelmann, 1996) is perhaps misleading, as is labelling it with ambient temperature. However, both labels are used in Figure 2, along with reference to receptor activation, to point out that the maximal states of vasoconstriction and vasodilation are primarily influenced by neuronal activity arising from thermoreceptors. Of

course, thermoreceptor activity is most often influenced by skin temperature, which can be altered by a number of factors, including ambient temperature (Mekjavic & Eiken, 2006) or water spraying (Savage & Brengelmann, 1996). The activity arising from thermoreceptors can also be influence by chemical compounds like menthol; one important distinction being that ambient temperature or water spraying can alter skin temperature and influence skin blood flow (Savage & Brengelmann, 1996), resulting in an inverse relationship between deep body and skin temperature. In contrast, menthol is thought to exert its influence on thermoreceptors without changing skin temperature; however, the influence that menthol exerts on most thermoregulatory responses is unknown. Only a few studies have assessed the influence of menthol on skin blood flow, mostly in the domain of psychophysiology. As a result, most of what is known comes from studies applying menthol to a small area of the skin, primarily the forearm, to the neglect of assessing the response at the systems level.

For example, Yosipovitch *et al.,* (1996) applied 10 % (620 mg · 100 $cm^{-2}$) menthol to the forearm and found no significant difference in skin blood flow after application. However, Wasner *et al.,* (2004) showed an increase in skin blood flow following application of 40 % menthol (3,200 mg · 100 $cm^{-2}$) to the forearm. Similarly, Namer *et al*., (2005) found an increase in skin blood flow following application of 40 % menthol (640 mg · 100 $cm^{-2}$) to the forearm, possibly owing to an inflammatory response. Johnson *et al.,* (2009) also found an increase in forearm skin blood flow following application of 3 % menthol (and 25 % ethanol, volume unspecified). Conversely, Olive *et al.,* (2010), observed a reduction in forearm vascular conductance after applying either 3.5 % menthol gel (17.5 mg · 100 $cm^{-2}$) or ice to the forearm. Unfortunately, this study did not benefit from a Control condition, so the cooling influence of the gel that was used to suspend the menthol in solution could not be determined. Further research is required to clarify the influence of menthol on vasomotor function and thermoregulation, particularly in the thermoneutral zone.

***Body temperature regulation during exercise***

If mean body temperature continues to increase, such as when exercise is undertaken, the capacity of the vasomotor response to regulate it is quickly surpassed. In this case, the autonomic response of sweating is initiated, but mean body temperature is still the regulated variable. Franks *et al.,* (1996) demonstrated this by having participants, who wore air perfused suits, walk on a treadmill in an ambient temperature of 23 °C until their deep body and skin temperatures plateaued. Participants were asked to complete three sub-

maximal exercise bouts; once with warm air circulating through the suit (30 °C), another with cool circulating air (20 °C), and a Control condition with no circulating air. The results showed that the cool air circulating condition had a strong inverse relationship between deep body and skin temperature, as did the warm circulating air condition but to a lesser extent; in all conditions mean body temperature remained constant.

Although mean body temperature is likely the regulated variable during rest (Savage & Brengelmann, 1996) and exercise (Franks *et al.,* 1996), the proportional neuronal input arising from thermoreceptors within skin and deep tissues of the body will differ in each case. For example, the input from deep body thermoreceptors (*i.e.* rectal temperature) in the study by Franks *et al.,* (1996) accounted for 93 % of the mean body temperature during exercise across the conditions. In contrast to this, Savage and Brengelmann (1996) showed that skin temperature was the main input influencing thermal balance in the thermoneutral zone, which suggests that the proportional input from skin thermoreceptors was greater than that from deep thermoreceptors, at least during rest in the TNZ.

When mean body temperature is calculated from a combination of skin and deep body temperature recordings, its measured value fluctuates. The zone in which it fluctuates without activating the mechanisms for evaporative heat loss (sweating) or metabolic heat production (shivering) is referred to as the thermoeffector threshold zone (or inter-threshold zone) (IUPS Thermal Commission, 2001). Figure 3 shows how mean body temperature is regulated in this inter-threshold zone, whereby changes in vasomotion exert controlling influence within limits, beyond what must be regulated by sweating or shivering (Werner *et al.,* 2008, pp. 332; Mekjavic & Eiken, 2006). The work undertaken in this thesis is primarily concerned with the sweating mechanism.

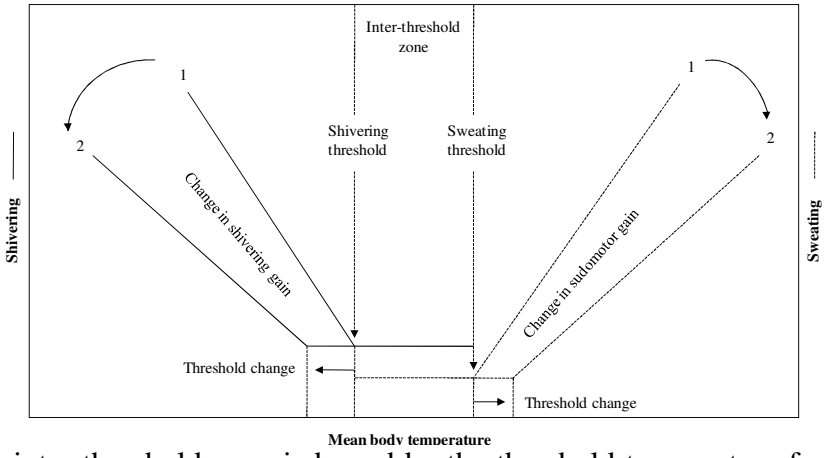


**Figure 3.** The inter-threshold zone is bound by the threshold temperature for the onset of shivering and sweating (adapted from Werner *et al.,* 2008; Mekjavic & Eiken, 2006).

At present, the central pathway mediating the increased sympathetic cholinergic outflow to sweat glands is not known (Morrison & Nakamura, 2011) but it is probably an integrated response that incorporates afferent signals from the deep body and periphery *i.e.* mean body temperature. Specifically, when mean body temperature exceeds the sweating threshold, which is usually 0.2 °C to 0.5 °C above the resting state, the onset of sweating can be observed (Taylor *et al.,* 2008). For this reason, sweating is most often assessed according to the mean body temperature associated with its onset, but also according to the sensitivity of the response; that is, the slope derived from the change in sweating over the change in mean body temperature, otherwise referred to as the gain. The onset and gain of thermoeffectors can be changed in the presence of thermal (*i.e.* ambient temperature), and non-thermal factors (*i.e*. plasma osmolality, blood glucose, age, fitness) (Mekjavic & Eiken, 2006). Further, the onset and gain of thermoeffectors can also change in response to repeated stimulation, so long as the stimuli are of sufficient strength to perturb homeostasis in the first place (referred to as a forcing function).

In one of the few studies to assess the influence of menthol on body temperature regulation and thermoeffector function during rest and exercise, Kounalakis *et al.,* (2010) asked participants to exercise on a cycle ergometer at 60 % of their $\dot{V}O_{2peak}$ until they reached a rectal temperature of 38 °C, once with a 4.6 % (4.6 g in 100 mL) menthol sediment over their whole body (27.5 mg · 100 $cm^{-2}$), and once without (NB. There was no 'water-only' Control condition). Kounalakis *et al*., showed that participants' deep body temperature increase more quickly after menthol spreading; also, the time of sweating onset was delayed, along with the change in rectal temperature required to initiate sweating. The difference in forearm and finger tip temperature, taken as an index of vasoconstriction (House & Tipton, 2002), was also greater, indicating a lower skin blood flow and a delay in the onset of vasodilation for the first 10 minutes of exercise.

A long standing theory first described by Sherrington (1906) and later re-emphasised by Bazett (1949) and Bligh (1998) asserts that the afferent fibres carrying information to the heat loss centre in the hypothalamus might have collateral pathways innervating the heat storage centre, and vice versa. The theory supposes that activation of one centre inhibits the other in a process referred to as reciprocal cross inhibition (RCI). Kounalakis *et al*., (2010) referred to this theory to explain the observation that exercising participants stimulated by menthol showed a withdrawal of the thermoeffectors for heat dissipation (*i.e.*

vasodilation and sweating). However, given that the pathways mediating the increase in sympathetic cholinergic outflow to sweat glands are presently unknown (Morrison & Nakamura, 2011), other theories might explain the results. For example, while the cumulative neuronal information arising from thermoreceptors within the body was sufficient to induce sweating in the Control condition, the added neuronal input arising from menthol-mediated activation of the TRPM8 cold receptors may have reduced the drive to initiate sweating after its integration in hypothalamic structures, independent of any RCI processes. Also, other mechanisms may explain the withdrawal of sudomotor function observed by Kounalakis *et al.,* (2010); indeed, it may have been suppressed by mechanical processes. For example, the menthol sediment, which was spread over the whole body and absorbed into the skin, may have contributed to swelling and blockage of the sweat duct in a process referred to as hidromeiosis (Werner *et al.,* 2008). It should be noted however, that Kounalakis *et al.,* (2010) did not spread menthol directly underneath the sweat capsule (7 $cm^2$), so it is perhaps unlikely that the measured withdrawal of sudomotor function was due to any local effect of hidromeiosis, but it raises the possibility that whole body sweat production may have been lowered. Lastly, Kounalakis *et al.,* (2010) used a dose of menthol that was approximately 15 times larger than the dose used by most commercial companies (*i.e.* 1.6 mg vs. 27.5 mg · 100 $cm^{-2}$), therefore it is not clear whether the heat storage response described by those authors applies to all menthol doses.

**Menthol and perception**

Although there is a large body of research describing menthol's perceptual influence, most studies are psychophysical in nature and only assess the perceptual response to small applications on the forearm of resting participants. Few studies have applied menthol to larger body surface areas, especially during exercise, so its influence on more global measures of perception, like thermal comfort or perceived exertion, is not well understood. In this section the studies assessing menthol's perceptual influence are reviewed, and an attempt is made to link this with what is known about the underlying mechanisms that influence the more global perceptions of thermal sensation and thermal comfort, irritation, and perceived exertion. An attempt is also made to clarify menthol's influence by dose, duration of effect, area of application, and individual difference where possible.

***Thermal sensation***

Menthol has long been known to enhance sensations of coolth when it comes in contact with the skin (Anonymous, 1924). But it was not until 1951 that the underlying mechanisms received some clarification. Specifically, electrophysiological studies undertaken by Hensel and Zotterman (1951) demonstrated that feline cold receptors which do not normally discharge at 40 °C will elicit a strong discharge following menthol application. Later commenting on the perceptual consequences for humans, Hensel (1981, p.32) stated that menthol '*elicits cold sensations at otherwise indifferent skin temperatures'*. Numerous studies have since confirmed Hensel's assertion, and further explored the perceptual influence of menthol in humans.

The smallest dose of menthol reported to elicit cool sensations is equivalent to 5 mg · 100 $cm^{-2}$ (applied to the forearm) (Watson *et al.,* 1978). Aside from this, most studies have used a much larger dose. For example, Green (1992) applied 60 mg · 100 $cm^{-2}$ and 120 mg · 100 $cm^{-2}$ to the forearm, Schlader *et al.,* (2011a) applied 500 mg · 100 $cm^{-2}$ to the face, Yosipovitch *et al.,* (1996) applied 620 mg · 100 $cm^{-2}$ to the forearm, Namer *et al.,* (2005) applied 640 mg · 100 $cm^{-2}$ to the forearm, and lastly, Wasner *et al.,* (2004) applied 3,200 mg · 100 $cm^{-2}$ menthol to the forearm. Green and Schoen (2007) also observed cool sensations after a 10 % menthol (forearm), but the volume was un-specified.

The influence menthol exerts over thermal sensation is thought to be dose dependent, but it is not clear how long the perceptual cooling lasts at a given dose. In one of the few studies to assess a range of menthol doses, Watson *et al.,* (1978) found that the dose of menthol (placed on the forearm) required to elicit sensations of coolth ranged from 5 to 1000 mg · 100 $cm^{-2}$ amongst 50 participants tested: 32 noted cool sensations when exposed to doses from 20 to 100 mg · 100 $cm^{-2}$, while six required more than 250 mg · 100 $cm^{-2}$ to notice coolth. Unfortunately, the duration of the cooling effect by doses was not quantified; however, Watson *et al.,* noted that '*the cooling effect…is rarely recorded for more than 15 minutes'* (Watson *et al.,* 1978, p.195). Yosipovitch *et al.,* (1996) were perhaps more precise when they applied 10 % (620 mg · 100 $cm^{-2}$) menthol to the forearm of 18 resting participants: 12 noted cool sensations that lasted 32 minutes on average (but ranged from 5 to 70 minutes). It is interesting that the dose used by Yosipovitch *et al.,* (1996) was at least six times greater than the dose that Watson *et al.,* (1978) found most individuals responded to (*i.e.* 32 out of 50 responded to 20 mg to 100 mg · 100 $cm^{-2}$ of menthol), yet the mean

cooling effect only lasted twice as long in the study by Watson *et al*.,. It is not clear whether the decay in thermal sensation over time follows from absorption of menthol in the skin and its subsequent clearance into the blood (Martin *et al.,* 2004), or whether other factors may interact to quicken its diminution, such as receptor adaptation, or the rise in mean body temperature and perceived exertion accompanying exercise.

The relationship between menthol dose and the perceived intensity of cooling also requires clarification. Although numerous studies have reported thermal sensation in response to various doses of menthol, different scales are often used to quantify perception, so it is difficult to establish clear relationships from the findings of published studies. However, Green (1992) applied menthol to the forearm in either 5 % (60 mg · 100 $cm^{-2}$) or 10 % (120 mg · 100 $cm^{-2}$) doses before cooling or warming the skin. He found that a doubling of the dose did not coincide with a doubling of perceptual cooling; suggesting the effect of menthol was nearing saturation at the lower dose.

It also remains to be clarified whether some body regions are more sensitive to menthol than others. Watson *et al.,* (1978) have suggested that the eye, mouth and nasal regions are most sensitive, and the soles of the feet and palms least sensitive, citing the thickness of the outer most layer of the epidermis as the main determinant of sensitivity. But, this assertion was not based upon empirical investigation and further research is required to determine whether some body regions are more sensitive to menthol than others.

To date, only one published study has assessed the influence of menthol on global thermal perceptions. Schlader *et al.,* (2011a) evaluated the independent roles of thermal perception and skin temperature in guiding work-rate by allowing participants to exercise at a fixed rating of perceived exertion, while undergoing either face cooling, face warming, or simulated face cooling (8 % menthol gel, 500 mg · 100 $cm^{-2}$), or simulated face warming (0.025 % capsaicin cream), or during a Control condition where their face was left alone. In this design, both face cooling and menthol improved thermal sensation and comfort, both of which lead to higher power outputs and longer exercise duration. But because the exercise protocol was fixed to a predetermined level of perceived exertion, rather than to a percentage of ones' maximal power output, work-rate (and metabolic heat production) was allowed to differ during each test. It is therefore difficult to fully separate the perceptual influence of menthol from the perceptions arising from different work-rates and metabolic

heat production. Future research is required to assess the perceptual influence of menthol during fixed rate exercise, when metabolic heat production is controlled.

***Irritation***

That menthol gives rise to sensations of irritation is not new; indeed, psychophysical studies applying a range of menthol doses to the forearm (620 to 3,200 mg · 100 $cm^{-2}$) consistently reported irritation, primarily sensations of burning, in addition to cool sensations (Green & Schoen 2007; Namer *et al.,* 2005; Wasner *et al.,* 2004; Yosipovitch *et al.,* 1996). This is probably because up to 50 % of primary neurons that respond to cold and menthol also have the noxious heat receptor TRPV1 (McKemy *et al.,* 2002); therefore, Green (2004) has suggested that some of the neurons that have TRPM8 may also project to the nociceptive pathway rather than, or along with, the cold pathway. It is interesting to note that Green and Schoen (2007) showed that both the cool sensations and perceived irritation induced by menthol (10 %, volume unspecified) applied to the forearm could be suppressed by dynamic contact (*i.e.* touching a thermode to the skin). These findings indicate that menthol stimulates the same afferent fibres that are responsible for the mild irritation observed when cooling the skin by just 2 °C (Green & Akirav, 2010).

As with thermal sensation, there seems to be a large individual variation in the sensation of irritation. For example, Yosipovitch *et al.,* (1996) applied 10 % (620 mg · 100 $cm^{-2}$) menthol to the forearm of 18 resting participants, but only eight complained of burning sensations, which lasted up to 40 minutes (seven of these also perceived a cool sensation). Similarly, Namer *et al.,* (2005) applied 40 % menthol (640 mg · 100 $cm^{-2}$) to the forearm of ten resting participants, seven of which noted pain that reached a peak value 16 minutes after menthol exposure. Lastly, Wasner *et al.,* (2004) applied 40 % menthol (3,200 mg · 100 $cm^{-2}$) to the forearm of ten resting participants, eight of which reported painful sensations. Of these, seven reported burning sensations, and one reported a 'dull' pain. The pain peaked eight minutes after the menthol exposure. It is not clear whether sensations of irritation, like those of thermal sensation, diminish as a result of biological menthol clearance to the blood (Martin *et al.,* 2004), or as a result of receptor adaptation.

It has been suggested that cool sensation varies directly with menthol dose, as does irritation, but cool sensation seems to vary less than irritation (Cliff & Green, 1994). Specifically, Cliff and Green (1994) have noted that doses of 0.03 % menthol preserve cool sensations but reduce the irritation caused by a 0.3 % menthol solution, at least in the oral

cavity. These findings suggest that by reducing the dose of menthol in a given solution, it may be possible to minimise sensations of irritation, whilst maintaining cool sensations.

***Thermal comfort***

To date, the work by Schlader *et al.,* (2011a) is the only published study to assess thermal comfort in response to menthol exposure. In it, participants felt more thermally comfortable when a large dose of menthol was spread over the face (500 mg · 100 $cm^{-2}$) during exercise in a cool environment (20 °C, 50 % rh), compared to a Control condition when the face was left alone. But menthol did not improve thermal comfort as much as actual face cooling using a fan. This suggests that the perceptual cooling induced by menthol was not exactly comparable to face cooling; some other factor was preventing an overall improvement to thermal comfort in the menthol condition. This highlights that thermal comfort is a multidimensional construct that is influenced by many factors. The following section attempts to highlight some of these factors.

In the domain of human applied environmental physiology, temperature perception is most often discussed in terms of 'thermal sensation' and 'thermal comfort', whereby the former describes the quality and intensity of a temperature stimulus, and the latter refers to the level of comfort resulting from that temperature stimuli; but more specifically, the IUPS Thermal Commission (2001) defines thermal comfort as subjective indifference to the thermal environment. In any case, thermal comfort is considered a higher order function than thermal sensation because greater processing is necessary to determine whether a given thermal stimulus is either pleasant (comfortable) or unpleasant (uncomfortable) (Rolls, 2010). It may not be surprising to learn that the areas in the brain where subjective experiences of pleasantness arise as a result of thermal stimuli have been found to differ from those areas where the physical characteristics of the thermal stimuli are processed (Rolls, 2008). For example, using fMRI in humans, Rolls *et al.,* (2008) correlated neural activation with ratings of pleasantness or unpleasantness in response to warm (41 °C) and cold (12 °C) hand stimulation. Activations in the mid-orbitofrontal and pregenual cingulate cortex and the ventral striatum were correlated with pleasantness. Activations in the lateral and anterior parts of the orbitofrontal cortex were correlated with unpleasantness, and activations in the somatosensory cortex and ventral posterior insula were correlated with the intensity of the thermal stimuli, but not with its pleasantness. By having one area of the brain tending to the reward aspects of thermal stimuli *i.e.* whether it is pleasant or not, and

another to the physical characteristics of that stimuli, Rolls (2010) contends that it becomes possible for humans to improve their comfort by modifying their behaviour (such as altering pace and metabolic heat production), while still tending to the important physical characteristics of the thermal stimuli. Building upon this notion, Rolls (2010) suggests that both warm seeking and cold avoidance behaviour *i.e.* thermoregulatory behaviour, can be thought of as goals for actions that are hard-wired within each of us to ensure survival.

Of course, a warm stimulus is not always considered pleasant, nor is a cold stimulus always unpleasant. Indeed, a cooled individual will report pleasure when stimulated with moderate heat, and displeasure with cold, while the opposite occurs when an individual is warmed (Cabanac *et al.,* 1972). Cabanac coined the term 'allesthesia' to describe this. Bringing together Cabanac's notion of allesthesia with Rolls' (2010) fMRI findings, we perhaps gain insight into the central structures that influence behaviour, but more importantly, we are reminded that pleasure (or comfort) is most easily obtained by restoring homeostasis (positive allesthesia), and lost by moving away from it (negative allesthesia). In this view, the question of what drives thermal comfort, or pleasure, in any given moment could simply depend upon how far one is from homeostasis; but is this homeostasis based upon perceptual or physiological variables? With this question in mind, researchers have sought to clarify the factors that influence thermal comfort.

When individuals are sat at rest in either a warm or cold room, and are given the option of improving their comfort by moving to a room that is either warm or cold, the main input that influences their decision is skin temperature (Schlader *et al.,* 2009). Alternatively, when participants are allowed to freely adjust the inlet temperature of their own water perfused suit so as to maintain thermal comfort during rest and mild intermittent exercise in a cold environment, it seems that mean body temperature primarily determines thermal comfort (Flouris & Cheung, 2009). In an effort to clarify the respective influence of deep body and skin temperature over thermal comfort, Frank *et al.,* (1999) used a water controlled mattress to alter skin temperature in isolation of deep body temperature, while the infusion of cold intravenous fluid cooled deep body temperature in isolation of skin temperature. The authors found that both deep body and skin temperature contributed equally, and individually, to thermal comfort.

Other studies have questioned whether some areas of the body exert greater influence over thermal comfort than others. Cotter and Taylor (2005) passively warmed individuals in a

climate chamber whilst they wore a water perfused suit, which allowed cooling of specific skin regions in isolation of other regions. It was found that face cooling was more effective in reducing thermal discomfort (and sweat rate) than any other area of the body. Similarly, Nakamura *et al.,* (2008) exposed participants to a warm or cool environment while they wore a water perfused suit, which allowed warming and cooling by body region. The authors found that during the heat exposure, face cooling influenced thermal comfort more than chest, abdomen or thigh cooling. But during cold exposure, chest and abdomen warming improved comfort the most. In the previously mentioned study by Schlader *et al.,* (2011a), both face cooling and menthol exposure improved thermal sensation and comfort compared to the Control condition, and led to higher power outputs and longer exercise duration, whilst facial warming had the opposite effect, possibly by enhancing inhibitory signals arising from warm thermoreceptors within the skin, which acted to lower pace. It also seems that increasing skin wettedness reduces thermal comfort, but the legs and arms appear to lose comfort more quickly than the front and back of the torso (Fukazawa & Havenith, 2009).

It should be noted that during exercise, a number of factors are introduced which influence thermal comfort that are not present during rest. For one, metabolic heat production increases, which increases the neuronal input from warm thermoreceptors within the body. But exercise in the heat causes more discomfort than the same exercise in cool conditions (Maw *et al.,* 1993); however, it is important to note that the former also increases sensations of warmth, perceived exertion, skin temperature, and cardiovascular strain. During exercise, individuals are also willing to accept a greater level of discomfort compared to resting conditions, at least with regards to the reduction in comfort associated with skin wettedness (Nishi & Gagge, 1977; Fukazawa & Havenith, 2009). Although it is not clear why this shift in acceptance occurs, it may be due to a change in expectation (*i.e.* participants expect to feel some discomfort during exercise), or perhaps the perception of effort arising from muscular and cardio-respiratory activity 'drown-out' sensations of discomfort arising from wet skin.

From these studies it becomes clear that numerous factors influence comfort. These factors include, but are not limited to: deep body, mean and local skin temperature, the rate of change of skin and deep body temperature, and thermoeffector function (De Dear, 2011). In addition, the area of skin exposed, stimulation history, experimental conditions (site of

testing, skin condition) and of course, individual difference (previous experience, ethnicity, sex, and stimulus intensity) are also important factors that influence our behavior (Schepers & Ringkamp, 2008). Unfortunately, the relative contribution of each factor to comfort requires further study.

Adding more complexity, psychological perspectives contend that an individual's sense of comfort is influenced by the match between one's expectations about the climate and what actually exists (De Dear & Brager, 2002). Further, these expectations are influenced by their interaction with the thermal environment (perceived thermal control) and past thermal experiences (Auliciems, 1981). Clearly, one's thermal comfort and behaviour is influenced by factors beyond the biophysics of heat exchange.

***Rating of perceived exertion***

Dr. Gunnar Borg suggested that the single best indicator of an individual's physical strain during exercise is their own rating of perceived exertion, or RPE (Borg, 1982). Accordingly, RPE exerts considerable influence over exercise pace (Tucker, 2009). During maximal exercise, RPE is most influenced by physiological factors; indeed Borg constructed the scale to grow linearly with work-load, and thus with heart rate and oxygen consumption (Borg, 1982). But RPE is also influenced by sensory input from other physiological systems within the body (Borg 1982), and during sub-maximal exercise, a number of other factors, both physiological and perceptual influence RPE and pace. For example, Nybo and Nielsen (2001) showed that RPE is highly associated with lowered cerebral function, which primarily accounts for reductions in work-rate during prolonged exercise in the heat. But RPE is also influenced by skin temperature (Tucker *et al.,* 2006; Schlader *et al.,* 2011b) and thermal perceptions (Schlader *et al.,* 2011a). In fact, research supports the assertion that exercise performance can be modified by various psychological interventions and strategies ranging from music (Boutcher & Trenske, 1990; Barwood *et al.,* 2009) to psychological skills training (Barwood *et al.,* 2008). Several studies highlight the brains role in pacing and exercise performance (Noakes, 2011), and in the context of this thesis, raise questions as to whether menthol might enhance or help maintain exercise performance in the heat. But without knowing how menthol influences RPE and other more global measures of thermal perception during fixed rate exercise, it is difficult to speculate whether it will influence pacing strategy, so it is prudent to first investigate

menthol's perceptual influence during fixed work-rate exercise, when power output and metabolic heat production is controlled.

**Menthol and habituation**

The study by Schlader *et al.,* (2011a) suggests that work-rate can be increased simply by improving perception alone, and menthol may promote this response. These findings raise the possibility of using menthol as a cooling strategy for work or exercise in the heat. Such an intervention could be used entirely at the users' discretion, perhaps daily, however the influence of repeated-daily menthol exposure on perception is not clear and it is important to clarify whether cool sensations habituate with repeated use. Habituation is defined by the IUPS Thermal Commission (2001) as *a reduction of responses to, or perception of, repeated (constant) stimulation*. The influence of repeated menthol stimulation on perception has received little attention, and those studies which have been carried out separate menthol exposures by minutes, not hours or days (Cliff & Green, 1994; 1996).

The study by Cliff and Green (1994) assessed whether cool sensations and irritation habituate after repeatedly exposing participants to either 0.03 % or 0.3 % menthol (in the oral cavity), separated by one or five minutes. The authors observed sensitization of cool sensations in some, and desensitization in others, suggesting that there is large individual difference in the perception of coolth; however, irritation seemed to desensitize with repeated application in the majority of participants. These findings were confirmed in a later study by the same group (Cliff & Green, 1996). These observations may be explained by the work of Campero *et al.,* (2009), who showed that menthol activates a subclass of nociceptors (Type 2C fibres) along with the TRPM8 receptor; thus, explaining how the perception of one modality (*i.e.* irritation) can undergo modification, while the other (*i.e.* thermal sensation) might not. But more importantly, these findings suggest that repeated menthol exposure may result in an habituation of irritation, while preserving sensations of coolth. It is not clear whether these findings would be replicated if the time between exposures were increased to hours or days, rather than minutes.

Given the paucity of research in this area, studies assessing cold adaptation in humans might give clues about the repeated use of menthol on thermal sensation. For example, a single exposure to menthol is perhaps similar to a single cold exposure in that each gives rise to sensations of coolth. The distinction being that menthol achieves this by direct

stimulation of the TRPM8 cold receptor without changing skin temperature (McKemy *et al.,* 2002; Peier *et al.,* 2002), whilst a cold exposure achieves this sensation by first lowering skin temperature, which increases the firing rates of cold receptors and brings about cool sensations. With this distinction in mind, repeated exposure to either cold air (Makinen *et al.,* 2006; Leppaluoto *et al.,* 2001; Bruck *et al.,* 1976) or water (Smolander *et al.,* 2004; Golden & Tipton, 1988) have been shown to cause an habituation of cool sensations and/or thermal discomfort. These findings suggest that repeated exposure to menthol may result in an habituation of thermal sensation, but the influence of repeated menthol exposure is not clear, so further research is required to clarify if there is any habituation in the initial perceptual responses to menthol.

Given the study by Kounalakis *et al.,* (2010), it seems that menthol applied to the skin surface represents a sufficiently potent stimulus to disturb thermoregulatory function following a single application; likely through modulation of the thermoeffectors. When a stimulus is strong enough to induce a change in homeostasis, adaptation theory suggests that the physiological impact resulting from the forcing function progressively changes after repeated exposures *i.e.* habituation (Tipton *et al.,* 2008). The most common effect of adaptation is a change in the effector threshold*; i.e.* a shift in the deep body temperature at which vasoconstriction, vasodilation, sweating and shivering begin (Tipton *et al.,* 2008). With no previous research outlining the physiological consequence of repeated menthol application, adaptation theory leads us to hypothesise an habituation in the heat storage response, probably owing to an upward shift in the deep body temperature associated with the onset of vasodilation and sweating, but this requires investigation.

**Conclusions**

It seems the global perceptual and thermophysiological impacts following a single exposure to a menthol-based spray are not well understood, and the responses following repeated exposures are completely unknown. Clarifying the mechanisms by which menthol exerts its influence over body temperature regulation and perception represents a fundamental line of enquiry, and given our limited understanding on the topic, it is prudent that before any menthol-based cooling solution is deployed as a 'performance intervention', a basic understanding is required of how such an intervention might exert its influence, and what side-effects might occur. The following section clarifies the methods employed in this thesis to investigate menthol's influence.

# Chapter 3

# General Methods

## Ethics

All studies undertaken in this thesis complied at all times with The Declaration of Helsinki, as adopted at the 18th World Medical Association (WMA) General Assembly, Helsinki, Finland, 1964 and last amended at the 59th World Medical Association General Assembly, Seoul, South Korea, 2008. All studies also complied with the Council of Europe (2005) and the convention on human rights and biomedicine concerning biomedical research; European Treaty Series No. 195, Strasbourg 25 January 2005. In addition, each study in this thesis received ethical approval from the BioScience Research Ethics Committee Review Board at Portsmouth University.

## Volunteer participants

Volunteer participants were recruited from the local University student population. Participants were between the ages of 18 to 29 years old, fit and healthy, with no history of heat illness. Written informed consent was obtained from each participant and kept on file. Each participant refrained from strenuous physical activity and alcohol consumption 24 hours prior to each exercise test and was asked to abstain from food and caffeinated beverages three hours before exercise on the day of data collection.

## Descriptions of the menthol sprays

In Study one, a menthol and ethanol water solution spray was compared to water spraying and no spraying. The solution was made by Physicool Ltd™ (London, U.K.) and contained 0.2 % (0.16 g) menthol and 20 % (16 g) ethanol, suspended in 80 mL of water. As menthol is not soluble in water, the ethanol held it in solution. When sprayed on the upper body, which represents 55 % of the total surface area (Yu *et al.,* 2010), this equated to 1.68 mg of menthol per 100 $cm^{-2}$ surface area for the average male with a body surface area of 1.76 $m^2$. In all other studies, the Control spray contained 3 g (3 %) of surfactants mixed in 100 mL of water, while the experimental sprays contained a dose of either 0.05 % (0.05 g) or 0.2 % (0.2 g) l-menthol in 100 mL of water. Each menthol solution also contained 3 g (3 %) of surfactants, which were used to hold menthol in the solution. When sprayed over the

entire upper body, 0.2 % menthol equated to 2.1 mg · 100 $cm^{-2}$, and 0.05 % menthol equated to 0.52 mg · 100 $cm^{-2}$.

All solutions were stored at room temperature and transferred into the environmental chamber 3 hours before testing; where they remained at chamber (testing) temperature until they were applied. All solutions were applied using a manual spray bottle. Spraying was chosen over other dispersion methods, such as spreading a menthol sediment cream (Schlader *et al.,* 2011a), or applying menthol with cotton balls (Kounalakis *et al.,* 2010) primarily due to its simplicity and transferability to a working or sporting scenario, but also because it allowed investigators to control the volume of solution applied and the location of spraying. Participants were given protective glasses and a mask during spraying to prevent any of the solutions coming into contact with the eyes, nose or mouth. To standardize the method of application, the same investigator sprayed the solutions during every test. The bottle was held 15 cm from the participant with each spray around the torso. The spray bottle was set to 'mist'. Each time the solution was sprayed, a surface area of approximately 15 cm x 15 cm was covered on the torso. Spraying was repeated until the entire upper body was covered evenly. The spray bottle was held closer during arm spraying to avoid wastage. 100 mL was sprayed on top of a long sleeve breathable shirt (100 % polyester), and over the entire upper body; approximately 30 mL on the back and front torso respectively, and approximately 20 mL on the left and right arms respectively.

**Physiological measurements**

***Rectal Temperature***

Rectal temperature ($T_{re}$) was measured using a rectal thermistor that was self-inserted 15 cm beyond the anal sphincter (Grant Instruments [Cambridge] Ltd., Royston, UK). The rectal thermistor has a reported accuracy of 0.1 °C. Between participants each rectal thermistor was sterilized in solution for a minimum of 1 hour (Haztabs, Edenbridge, Kent, U.K). The accuracy of rectal thermistors was assessed prior to each experiment using a small heated water bath (Grant Instruments [Cambridge] Ltd., Royston, UK) that changed temperature in 0.5 °C increments within the range expected in the experiment (36.5 °C to 39.5 °C). The temperature of the water bath was monitored with a thermometer (Digitron thermometer T600, RS calibration, UK) certified to British standards BS EN ISO 9001: 2008. Thermistors were not used if they deviated more than 0.1 °C from the calibrated thermometer reading.

***Skin Temperature***

Participants were instrumented with skin thermistors (Grant Instruments [Cambridge] Ltd., Royston, UK) secured by micropore tape (Tegaderm™ Film, 3M, U.K.), at: left chest ($T_{chest}$), right scapula ($T_{scap}$), left lateral biceps ($T_{bicep}$), back of the left hand ($T_{hand}$), left hamstring ($T_{ham}$), right vastus mediais ($T_{vm}$), right tibialis anterior ($T_{ant}$), and atop the right foot ($T_{foot}$). The contact area of each thermistor was cleaned with an alcohol swab prior to use. Skin thermistors have a reported accuracy of 0.2 °C, which was assessed as per rectal thermistors, but in the range of 33 °C to 40 °C.

***Infra-red thermography***

A thermal imaging camera (A320 series, ThermaCAM™, FLIR systems, Kent, UK) was used to capture images in the infra-red spectral range of 7.5 μm to 13 μm, with a temperature range from minus 20 °C to 120 °C and an accuracy of 2 %. At 25 °C the camera has a sensitivity of 0.07 °C, and a focal plane array containing 320 x 240 pixels. Thermal images were analysed using proprietary software (Researcher 2.9, FLIR systems, Kent, UK), which allowed the user to select an area of interest *i.e.* chest/front torso (from the nipple line to the umbilicus), or back (from the shoulders to the height of the umbilicus), and obtain a mean surface temperature from that area.

***Skin blood flow***

Skin blood flow (SkBF) was measured using a laser Doppler blood flow monitor (MoorLab, Moor Instruments Ltd., Axminster, UK). Laser light leaves the probe and enters the skin where it contacts red blood cells in the cutaneous circulation and is reflected back towards the probe. The Doppler frequency shift of the reflected laser light indicates the velocity of red blood cells and the intensity of that reflected light indicates the concentration of blood cells. The product of these two (concentration and velocity) give an estimate of flux. Flux measurements were made at four sites; medial thigh, index finger, medial forearm, and at the left side of the lower back using a fiberoptic probe lightly affixed to the skin with tape (Tegaderm™ Film, 3M, UK). The probes were calibrated before each use against a standard reference (flux standard, cod PFS) provided by the manufacture. The standard uses the Brownian motion of polystyrene microspheres in water to produce the reference signals. During testing, data were sampled using an analogue to digital converter (Powerlab; AD Instruments, Ltd., UK) every second on a personal computer. All data were imported into an excel worksheet and averaged for each minute.

***Sweat rate - Ventilated sweat capsule***

Sweat rate was measured using ventilated sweat capsules (Q-Sweat Quantitative Sweat Measurement System, Model 1.0, WR Medical Electronics Co., Minnesota, USA). Each Q-Sweat sensor was calibrated by the manufacturer. Sweat output measurement is based upon direct vapor pressure calculations performed using WR TestWorks Software, version 2.2. Each measurement area was 0.787 $cm^2$ with a dry air flow rate of 0 to 100 $cm^3 \cdot min^{-1}$. Sweat volume calculations were derived from rate and time, have an accuracy of 5 %, repeatability is 5 %, and sensitivity is 10 nanoliters (nL). Sweat rate measurement ranged from 0 to 1000 nanolitres per minute ($nL \cdot min^{-1}$). Measures were converted to $mL \cdot m^2 \cdot min^{-1}$.

***Exercise testing***

Exercise testing allowed for the precise quantification of a participant's peak power output ($PO_{peak}$). By having participants then exercise at the same percentage of their peak power output during each test, rather than the same absolute work load, it was hoped that the level of physiological strain would be more comparable between participants during exercise. To this end, each participant performed an incremental test until exhaustion on a Monark cycle ergometer (Monark Exercise AB, Sweden) starting with a 5 minute warm-up period where they cycled at 60 rpm with a 1 kg carriage weight, equating to 60 W. Each subsequent minute, work-rate was increased by adding a 0.5 kg load to the carriage, which is equivalent to an increase of 30 W, until the participant was no longer able to maintain a pedaling frequency of 60 rpm. Peak oxygen uptake ($\dot{V}O_{2peak}$) was defined as the highest value attained during the test as analyzed retrospectively from the gas collected in the Douglas bags (60 second collection per bag), provided that the participant also attained either their age-predicted maximal HR (220 minus age) during the test, or they reached a respiratory exchange ratio (RER) of greater than 1.10 (Hale, 2003).

***Oxygen consumption***

The Douglas bag method was employed to measure pulmonary gas exchange because this is considered the gold standard measurement (Winter, 2007). Expired air was collected in individually labeled Douglas bags, analysed for their composition of carbon dioxide ($CO_2$) and oxygen ($O_2$) (Servomex Analyzer Series 1400, Servomex®) and evacuated to determine the volume of gas collected using a dry gas meter (Harvard Apparatus Ltd., UK). The volume of Oxygen ($\dot{V}O_2$) and carbon dioxide ($\dot{V}CO_2$), and the fraction of expired oxygen ($F_{EO_2}$) and carbon dioxide ($F_{ECO_2}$) was calculated. The gas temperature

was measured using a digital thermometer (Checktemp, Portugal) and the barometric pressure was recorded (Fortin, Russell Scientific Instruments, Norfolk, UK) to allow calculation of $\dot{V}O_2$ and $\dot{V}CO_2$ in STPD. All Douglas bags were checked for leaks prior to use. Expired gas samples were obtained for one minute during rest, and two minutes during exercise. All gas analysis equipment underwent a three-point calibration before use, whereby it was first calibrated against a gas with a known composition of oxygen (~ 15 %) and carbon dioxide (~ 5 %). The composition of oxygen (15 %) and carbon dioxide (5 %) in this calibration mixture represent the low and high expected values, respectively, that might be encountered with exercise. The gas analysis equipment was also calibrated against atmospheric oxygen (20.95 %) and carbon dioxide (0.03 %). The composition of oxygen (15 %) and carbon dioxide (5 %) in the atmosphere represented the high and low expected values, respectively, that might be encountered in resting participants during testing. The gas analysis equipment was also calibrated against nitrogen, which is inert, and as such served as a zero reference.

***Heart rate***

Heart rate (HR) was measured using a chest-strap heart rate monitor (Polar, Finland) and was recorded by the minute.

**Perceptual measurement**

Laminated visual analogue paper scales for thermal sensation (TS), thermal comfort (TC), rating of perceived exertion (RPE) and irritation (IRR) were held either in front of participants, or placed on top of the handle bars of the cycle ergometer, directly in front of participants throughout experiment. Using a washable marker, participants placed a straight line at the location that described their perception for each scale. The location of the mark was measured using a standard ruler (cm), or in the case of RPE, a mark was placed next to the verbal descriptor and associated number. After recording the participants score on a data collection sheet, the washable mark was erased. The Borg (1982) scale, ranging from 6 to 20, was used to assess rating of perceived exertion (RPE). The perceptual scales for thermal sensation and comfort, and irritation, are described below.

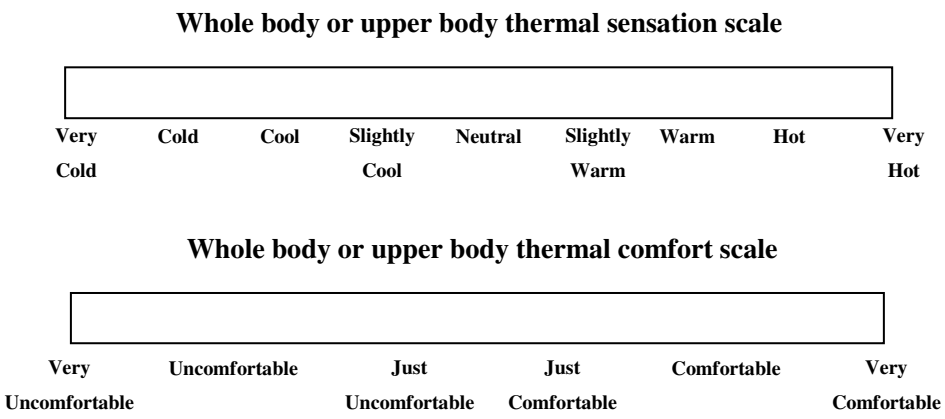


**Figure 4.** Thermal sensation and comfort scales (not to scale, adapted from Zhang, 2003).

As participants were to exercise in the heat they were likely to experience warm sensations and thermal discomfort, but after spraying they might feel cooler and greater thermal comfort. 20 cm bipolar scales for thermal sensation and thermal comfort (shown in Figure 4, but not to scale) were therefore used to measure the shifts between warm and cool sensations, and between thermal comfort and discomfort (Zhang, 2003).

In Study two, participants were asked to describe the quality of the irritation they experienced after menthol spraying. The construct of irritation is multidimensional and includes the descriptors listed below in Figure 5, which were proposed by Green (1992). To describe the quality of irritation, participants were asked to select as many or as few descriptors as they felt necessary to fully describe their perceived irritation.

- ***Burning*** – the sensation that commonly results from exposure to very high temperatures, skin abrasions, rug or floor burns, or chemical irritants such as alcohol. This may or may not be accompanied by thermal stimuli.
- ***Stinging/pricking*** – sharp sensations similar to those produced by an insect bite (other than itching) or by a pin-prick; may be constant (stinging) or intermittent (pricking).
- ***Itching*** – the sensation that causes the desire to scratch.
- ***Tingling*** – a lively "pins-and-needles" sensation.
- ***Numbness*** – the diffuse (fuzzy) sensation produced during the onset or offset of anesthetic (novocaine); not the complete absence of sensation.
- ***Ache*** – a dull, uncomfortable sensation that fluctuates in strength and is often difficult to localize.
- ***Pain*** – any sensation that "hurts"

**Figure 5.** Descriptors of the quality of irritation (adapted from Cliff & Green, 1994).

The main drawback of this method is that no information about absolute perceptual intensity can be obtained, and direct comparisons of descriptors between participants are meaningless (Green *et al.,* 1993). The most direct way to study perceptual differences

between participants and to obtain data on the absolute intensity of a sensation is to use a category scale. The main drawback of using a category scale is it only allows inferences to be made about the rank-order of the different sensations. To overcome these issues Green *et al.,* (1993) developed a scale of sensation magnitude with apparent ratio properties and called it the labeled magnitude scale (LMS) (shown below in Figure 6).

The LMS is a category-ratio scale with labeled intensity descriptors (Green *et al.,* 1993). The scale is bounded at the bottom by 'no sensation' and at the top by 'strongest imaginable sensation'. The key feature of the LMS is that its verbal descriptors (barely noticeable, weak, moderate, strong, and very strong) are placed quasi-logarithmically at locations along a straight line that are determined by estimations of their perceptual magnitudes (Green *et al.,* 1993). It has been suggested that evenly spaced descriptors may distort the measurement of perceptions like irritation because the verbal labels are not placed in order of their magnitudes as perceived by those experiencing sensations (Green *et al.,* 1993).

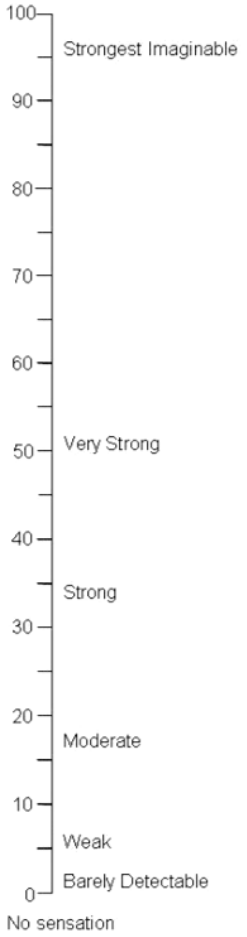


**Figure 6.** Labeled magnitude scale for irritation (Green, 1993) (not to scale)

The LMS is capable of generating ratio-level data in many sensory modalities, including sensations of irritation (Green *et al.,* 1993) and has been used to quantify sensory irritation attributable to menthol in numerous studies (Cliff & Green 1994; Green *et al.,* 1992; Green *et al.,* 2007; Green *et al.,* 2008). The authors of the LMS also claim that their scale shows good face validity because category scales always place verbal descriptors evenly, with 'moderate' in the middle, whereas the LMS placed moderate at 17 % of the full range of imaginable sensations. The authors suggest that most people would agree that 'moderate' sensation is not half as strong as the strongest imaginable sensation. Herein lies the key

difference between the LMS and category scales, with the latter not truly measuring the underlying perceptual dimension of intensity and the former being the best measure for providing high quality ratio level data on perceived intensity (Personal correspondence with Professor Green, 2008). A labeled magnitude scale (LMS) (Figure 6) was therefore used in the third and fourth studies to quantify the intensity of irritation resulting from menthol spraying.

***Thermal sensitivity testing***

Three familiarization tests of thermal sensitivity were undertaken prior to the start of testing in order to account for any learning effect (Golja *et al.,* 2003). Participants were tested for their sensitivity to detect warm temperature stimuli using a thermal sensitivity tester (Physitemp Instruments Ltd., New Jersey, USA). The system includes a water pump and tank unit (9.4 L capacity, PTU-110A), which is connected to a controller (NTE-2 A) by an in/out water tubing system. The controller is connected to a thermal plate (2" x 1 ¾" x 1 ¼" thick) by two additional water tubes. The thermal plate is mounted on a stage. The thermal plate has an accuracy of 0.1 °C at a rate of temperature change of 0.33 °C · $s^{-1}$. The plate has a temperature range of 0 °C to 50 °C. The mean (SD) adapting temperature of the thermal plate was 33 °C (0.1 °C) throughout testing.

The thermal plate was inverted and mounted on a guiding system so that it could be placed level on the volar side of the left forearm approximately 2.5 cm from the elbow joint, with the hand in a supinated position resting on a padded table. Participants were asked not to move their forearm for the duration of thermal sensitivity testing (approximately 10 minutes). Participants were instructed that a warm temperature stimulus would be presented to the skin through the thermal plate. Immediately after the presentation of the warm temperature stimuli, participants were instructed to report whether they perceived a change in the resting temperature of the plate and after each temperature change the plate was returned to the adapting temperature of 33 °C. If the participant perceived a change in the resting temperature, the next applied thermal stimulus was smaller. In the event that the stimulus was not perceived, the next stimulus was greater. Sham stimuli were intermittently initiated whereby no thermal stimuli were presented. The threshold for warm sensation was calculated as the average of the last 10 stimuli presented.

**Data acquisition**

All thermometry data were logged every minute by either a SQ 1000/1250 series Squirrel data logger (Grant Instruments Cambridge Ltd., Royston, UK) or an MSR-12 data logger, (MSR electronics GmbH, Switzerland). HR was recorded by hand on a data sheet every five or 10 minutes. Ventilated sweat capsule data were recorded four times per second, and then averaged by the minute. Laser Doppler data were recorded once per second, but as flux data can be highly variable within and between participants, attempts were made to smooth and normalise it. First, an average of the highest 60 values from the entire data set was taken to serve as a 100 % reference value. All data were then normalized to this 100 % value, and averaged by the minute. Data were then reported as their % change from their lowest point during testing, equating to a state of vasoconstriction, which normally occurred immediately after spraying. This allowed the investigator to observe the onset of vasodilation more easily.

**Calculations**

Mean skin temperature ($\overline{T}_{msk,}$ °C) was calculated using Ramanathan's formula in Study two, consisting of four skin sites. The number of sites was increased to eight, using Olesen's formula in Studies three and four, so as to more accurately estimate mean skin temperature.

$$\overline{T}_{msk} = 0.3T_{chest} + 0.3T_{arm} + 0.2T_{thigh} + 0.2T_{calf} \text{ (Ramanathan, 1964)}$$

$$\overline{T}_{msk} = (T_{chest} * 0.196) + (T_{scapula} * 0.209) + (T_{bicep} * 0.095) + (T_{hand} * 0.067) + (T_{hamstring} * 0.113) + (T_{thigh} * 0.098) + (T_{shin} * 0.142) + (T_{foot} * 0.082) \text{ (Olesen, 1984)}$$

Mean body temperature ($\overline{T}_b$, °C) was calculated using the formula by Burton, which places a greater weighting on skin temperature than other formulae *i.e.* Colin *et al.,* 1971; $\overline{T}_b = (T_{re} * 0.79) + (\overline{T}_{msk} * 0.21)$. Hence, Burton's formula better reflects the changes in $\overline{T}_b$ resulting from skin surface spraying during exercise in warm humid conditions.

$$\overline{T}_b = (T_{re} * 0.65) + (\overline{T}_{msk} * 0.35) \text{ (Burton, 1935)}$$

Thermoeffector function, specifically the onset of vasodilation or sweating, was identified as the time (minutes) when the response raised two SD above the cumulative mean score.

**Data analyses**

All data were tested for distribution normality using the Kolmogorov-Smirnov test for small sample size (six or less), while the D'Agostino and Pearson omnibus normality test was used for normality testing in larger groups. Specific statistical analyses will be briefly described in the methods section of each study. Values are means (SD) for parametric data, and median (range) for non-parametric data. A $P$ value $< 0.05$ was considered statistically significant. All statistical testing was performed using GraphPad Prism version 5.00 for Windows, (GraphPad Software, San Diego California USA).

# Chapter 4

# Study 1: An initial assessment of the influence of torso skin wetting with a menthol + ethanol solution on thermal perception and deep body temperature during exercise in warm, humid conditions

## Introduction

In warm, humid conditions, the thermal gradient between the skin and environment is reduced, as is the capacity for evaporative heat loss. These factors, along with an increase in metabolic heat production resulting from exercise can reduce work capacity and exercise performance (Rowell *et al.,* 1966). Thermoreceptors located within the body convey information about this accumulation of thermal energy to higher brain structures, and when mean body temperature rises uncontrollably, the cumulative integrated neuronal input is thought to eventually give rise to inhibitory signals that lower power output to protect the organism from heat injury (Nybo, 2010). Lessening the inhibitory signals during exercise in the heat may enhance, or help to maintain performance. Given the inhibitory signals seem to be accentuated by warm thermoreceptor activation (Tucker *et al.,* 2006; Schlader *et al.,* 2011a, 2011b), they might be attenuated by the cold receptor activation that follows skin cooling (Schlader *et al.,* 2011a); furthermore, skin cooling also improves thermal perceptions which may, in itself, enhance work-rate (Schlader *et al.,* 2011a).

For these reasons, UK Sport requested the Extreme Environments Laboratory at Portsmouth University to test a commercially available cooling solution spray (Physicool™, London, U.K; Energizer™) composed of 0.2 % menthol and 20 % ethanol (in 80 mL of water) in the run-up to the Beijing Olympics of 2008. Specifically, UK Sport questioned whether the combined menthol and ethanol spray could alleviate heat stress amongst athletes and support staff in Beijing. UK Sport was interested in the combined menthol/ethanol solution spray because it claimed to enhance evaporative heat loss from the skin and improve thermal perceptions in hot environments; however, it is not clear whether these products provide greater evaporative cooling or enhance thermal perceptions compared to water spraying alone, or no spraying at all. Before such products are recommended as ergogenic aids, these studies should be undertaken.

Recently, Mujika *et al.,* (2010) provided highly trained rowers with forearm sweatbands soaked in either a cooling solution (ethanol, menthol and water; Energizer™ Liquid Ice CosMedicals Inc. AG, Switzerland;) or water alone, (NB. no Control condition), during an indoor 2000 m self-paced time trial. The authors observed no significant difference in perceived exertion, time to finish, or pacing between the interventions. The evaporative cooling capacity of this intervention was limited because the surface area exposed to the solution was small (forearms only), but also because the sweat bands created an additional barrier to evaporative heat loss between the skin and the environment. Also, the influence of the ethanol/menthol solution on thermoregulation could not be assessed directly given the self-paced study design, which did not control metabolic heat production. Such interventions should be improved by applying the solution over a larger surface area to allow for greater heat exchange, and by replacing the cotton sweat band with a lightweight 100 % polyester breathable fabric garment to optimise the difference between the vapour pressure at the skin and the air, and thereby increase evaporative heat loss. Furthermore, the effectiveness of this intervention should be assessed during fixed work-rate exercise to control metabolic heat production. This raises the possibility of spraying a menthol/ethanol solution on breathable shirts that are commonly used in many sporting scenarios.

An 80 mL solution composed of 20 % ethanol (16 mL), 80 % water (64 mL), and menthol (0.2 %, or 16.8 mg) has the potential to remove 171.5 kilojoules (kJ) of thermal energy from the skin as it evaporates (14.7 kJ from ethanol and 156.8 kJ from water). Alternatively, 80 mL of water will remove 196.6 kJ, or 25 kJ more thermal energy than the 20 % ethanol + water spray. It is important to note that the ethanol component of the aforementioned solution will evaporate more quickly than the water component due to its lower latent heat of vaporisation, and herein lays the enhanced cooling potential of the 20 % ethanol solution. Specifically, at an ambient temperature of 21 °C and 60 % rh, one gram of ethanol will store 920 joules of thermal energy and evaporate in just above five minutes (Godts *et al.,* 2005). One gram of water, however, stores 2,450 joules, but takes 30 minutes to evaporate completely in the same environmental conditions (Godts *et al.,* 2005). Given that removing 3.47 kJ of thermal energy from 1 kg of human tissue will result in an average tissue temperature reduction of 1 °C (Burton, 1935), spraying 80 mL of 20 % ethanol + 0.2 % menthol + water has the potential to reduce the temperature of 1 kg of tissue by 2.3 °C · $min^{-1}$, or remove thermal energy at a rate of 8.2 kJ · $min^{-1}$, for the first five minutes after it is applied on the skin. Water spraying alone, however, has the

potential to reduce the temperature of 1 kg of tissue by 1.8 °C · $min^{-1}$, or remove thermal energy at a rate of 6.5 kJ · $min^{-1}$, for the first five minutes. The difference in cooling amounts to 1.7 kJ · $min^{-1}$, or 0.5 °C · $min^{-1}$ over the first five minutes. So, although the absolute capacity of the ethanol solution to remove heat through evaporation is 2.5 times less than water, it can remove heat more quickly, particularly in the moments after spraying. This suggests that the 20 % ethanol spray has the potential to enhance evaporative heat loss more than water when it is repeatedly applied. But it is not clear whether this translates into lower skin and rectal temperatures during exercise in the heat.

In addition to the evaporative cooling potential attributed to ethanol, menthol, also contained within said cooling solutions, '*elicits cold sensations at otherwise indifferent skin temperatures'* (Hensel 1981, p.32), but may also give rise to heat storage, perhaps owing to a withdrawal of sudomotor function and increase in vasoconstrictor tone (Kounalakis *et al.,* 2010). It is difficult to predict whether the theoretical improvement in evaporative cooling imparted by ethanol will outweigh the potential heat storage induced by menthol, and whether thermal perception will improve, or be impaired as a result. It remains unclear whether a menthol/ethanol/water-based cooling spray absorbed into breathable garments, with replenishment, may provide effective short and long term improvements in evaporative cooling and thermal perceptions.

## Aims and Hypotheses

The primary aim of this study was to assess whether a water spray containing 0.2 % menthol and 20 % ethanol could improve evaporative cooling and thermal perceptions over a water spray, or no spraying at all, during rest and exercise in a warm humid environment.

***Null hypotheses (NB. These hypotheses were formulated in 2008, prior to the work by Kounalakis et al., [2010], which described the menthol-mediated heat storage response)***

1. There will be no difference in rectal temperature between the 0.2 % menthol + 20 % ethanol spray, water spray, and no spray condition during rest or exercise.
2. There will be no difference in thermal sensation and comfort after 0.2 % menthol + 20 % ethanol spraying compared to water spraying or no spraying during rest or exercise.

## Methods

### *Participants*

Six volunteer participants with a mean (SD) age of 22 (4) years participated in this study.

***Procedure***

Participants completed three, two hour tests in warm, humid conditions (30 °C, 70 % rh); each test was split into four 30 minute periods. During the first 30 minutes of each hour, participants engaged in low intensity stepping exercise at a rate of 12 steps per minute onto a 22.5 cm box. The second 30 minutes consisted of seated rest. Both the environmental conditions and the stepping exercise were chosen to reflect the conditions and activity pattern that British Olympic support staff would likely encounter when supporting British athletes during the Beijing Olympics of 2008. The experimental timeline is shown below.

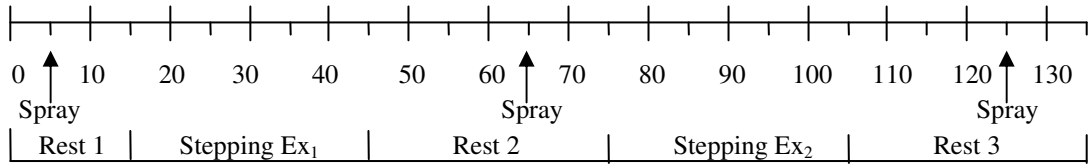


**Figure 7.** Study one experimental timeline

During each test participants were assigned, in a balanced three-by-six Latin square, to one of three different conditions consisting of long sleeve sports shirts (breathable 100 % polyester) sprayed with either 0.2 % menthol + 20 % ethanol (M/E), water alone (W) or an unsprayed dry shirt serving as a control (CON); otherwise participants wore shorts and trainers. The shirts were sprayed initially and replenished with 80 mL of either spray every 60 minutes. The spray frequency was set at 60 minute intervals to allow enough time to observe the influence of a single application of each spray, but frequent enough to evaluate whether repeated spraying of either solution could enhance evaporative heat loss from the skin during exercise in warm, humid conditions.

***Measurements***

Participants reported their whole body thermal comfort (TC), thermal sensation (TS) and rating of perceived exertion (RPE). Rectal temperature ($T_{re}$) was measured using a rectal thermistor. Skin temperature was measured at the right chest ($T_{chest}$), left scapula ($T_{back}$), and right forearm ($T_{forearm}$) using skin thermistors. An estimation of mean skin temperature was obtained using a thermographic camera, which captured images of the back and upper torso/chest. Heart rate (HR) was measured using a polar heart rate monitor.

***Analyses***

A two-way repeated measures ANOVA, by spray group and time (and interaction) assessed statistical significance of parametric data. Non-parametric data were analysed

using Friedman's one-way repeated measures ANOVA, and reported as median (range) values. The alpha level was set at 0.05, unless otherwise specified.

## Results

### *Environmental conditions*

Environmental temperature and relative humidity (rh) did not differ ($P > 0.05$). Mean (SD) dry, globe and wet bulb temperatures were 29.5 (0.1) °C, 29.6 (0.1) °C and 26.4 (0.6) °C respectively. Mean (SD) relative humidity was 68.5 (0.5) %.

### *Measures of work-rate*

During each resting period, the overall group mean (SD) HR remained at 74 (9.2) beats · $min^{-1}$, but increased to 93 (8.9) beats · $min^{-1}$ with each period of stepping exercise. A two-way repeated measures ANOVA showed a significant difference in HR over time ($P < 0.0001$), but not by spray group ($P > 0.05$); with an interaction observed between the two factors ($P < 0.05$); the interaction could not be located with *post-hoc* testing. RPE remained stable ('very light') during each phase of stepping exercise across all groups. Friedman's ANOVA showed no difference in RPE by spray group ($P > 0.05$). Median (range) RPE in CON, W and M/E averaged over both stepping phases were 8 (7 to 13), 8 (7 to 12) and 8 (6 to 16) respectively.

### *Rectal temperature*

Figure 8a shows median $T_{re}$ during each exercise and resting period, by spray group, Figure 8b shows the median $\Delta T_{re}$ over the same period. Friedman's ANOVA showed a difference by spray group ($P < 0.0001$) in both $T_{re}$ (Figure 8a) and $\Delta T_{re}$ (Figure 8b). *Post-hoc* testing of the absolute data indicated that W and M/E were significantly different from CON ($P < 0.05$). *Post-hoc* testing of the $\Delta T_{re}$ data indicated that M/E resulted in a greater $\Delta T_{re}$ compared to CON ($P < 0.01$) and W ($P < 0.001$), while W had a smaller $\Delta T_{re}$ than CON ($P < 0.05$).

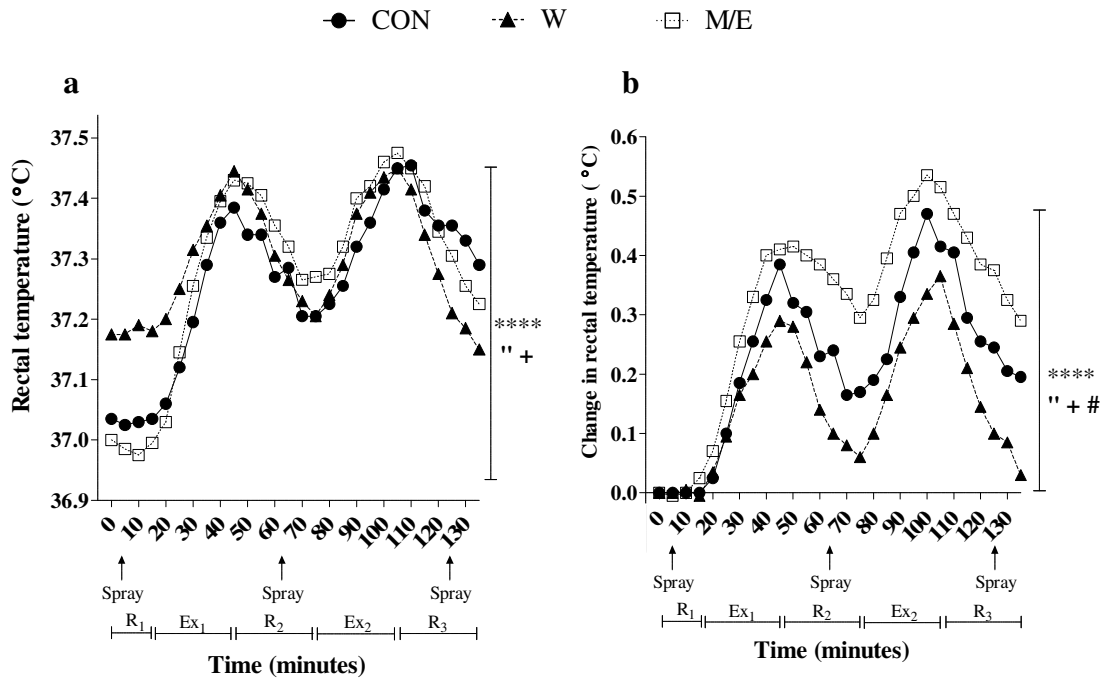


**Figure 8.** Median rectal temperature (a) and the median change in rectal temperature (b) during exercise and rest, by spray group ($n$ = 6). Significant difference (****$P$ < 0.0001) by spray group (⌶). *Post-hoc* test: Significant difference between W and M/E (#, $P$ < 0.05); between W and CON (**"**, $P$ < 0.05); between M/E and CON (**+**, $P$ < 0.05).

***Thermography***

Figure 9a shows mean surface temperature of the front torso and chest, taken with the infra-red thermal imaging camera, during exercise and rest, by spray group. A two-way ANOVA showed that front torso temperature differed over time ($P$ < 0.0001) and by spray group ($P$ < 0.0001), with an interaction ($P$ < 0.0001). *Post-hoc* testing showed that torso temperature remained lower in both W and M/E compared to CON ($P$ < 0.05), but there was no difference between W and M/E ($P$ > 0.05), except at the 70$^{th}$ minute, when M/E induced cooler skin temperatures than W and CON ($P$ < 0.05).

Figure 9b shows mean surface temperature of the back, during exercise and rest, by group. A two-way ANOVA showed that back torso temperature differed over time ($P$ < 0.0001) and by spray group ($P$ = 0.035), with an interaction ($P$ = 0.016). *Post-hoc* testing showed that back temperature remained cooler in W and M/E compared to CON ($P$ < 0.05), particularly at the start of $Ex_1$, but there was no difference in back temperature between W and M/E ($P$ > 0.05).

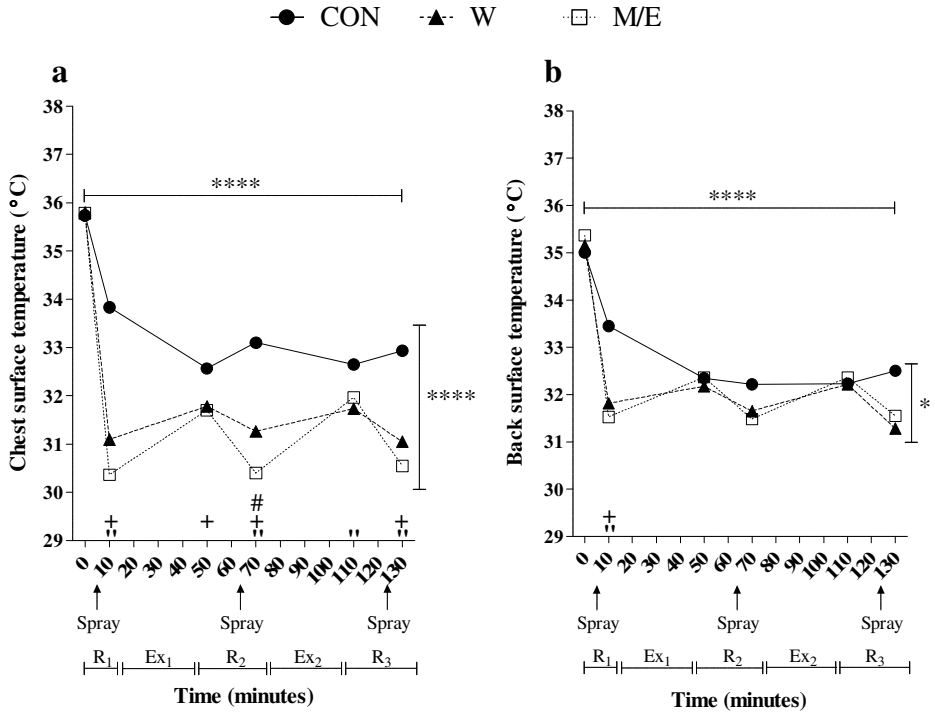


**Figure 9**. Mean surface temperature of the front (a) and back torso (b), taken with the infra-red thermal imaging camera, during exercise and rest, by spray group ($n$ = 6). Significant difference (*$P$ < 0.05; ****$P$ < 0.0001) by time (⊢⊣) and by spray group (⌶). *Post-hoc* test: Significant difference between W and M/E (#, $P$ < 0.05); between W and CON ('', $P$ < 0.05); between M/E and CON (+, $P$ < 0.05).

***Skin temperature at the chest, back and forearm***

Figure 10a shows median $T_{chest}$ during exercise and rest, by group. Friedman's ANOVA showed a difference by spray group ($P$ < 0.0001), and *post-hoc* testing showed that both W ($P$ < 0.001) and M/E ($P$ < 0.001) had lower temperatures than CON, but there was no difference between W or M/E ($P$ > 0.05). Figure 10b shows median $T_{back}$ during exercise and rest, by group. Friedman's ANOVA showed a difference in $T_{back}$ by spray group ($P$ < 0.0001), and *post-hoc* testing showed that both W ($P$ < 0.001) and M/E cooled the skin more than CON ($P$ < 0.001), but there was no difference in cooling between W or M/E ($P$ > 0.05). Figure 10c shows median $T_{forearm}$ during exercise and rest, by group. Friedman's ANOVA showed a difference in $T_{forearm}$ by spray group ($P$ < 0.0001) and *post-hoc* testing showed that W ($P$ < 0.001) and M/E ($P$ < 0.001) lowered forearm temperature more than CON. M/E lowered temperature more than W ($P$ < 0.05).

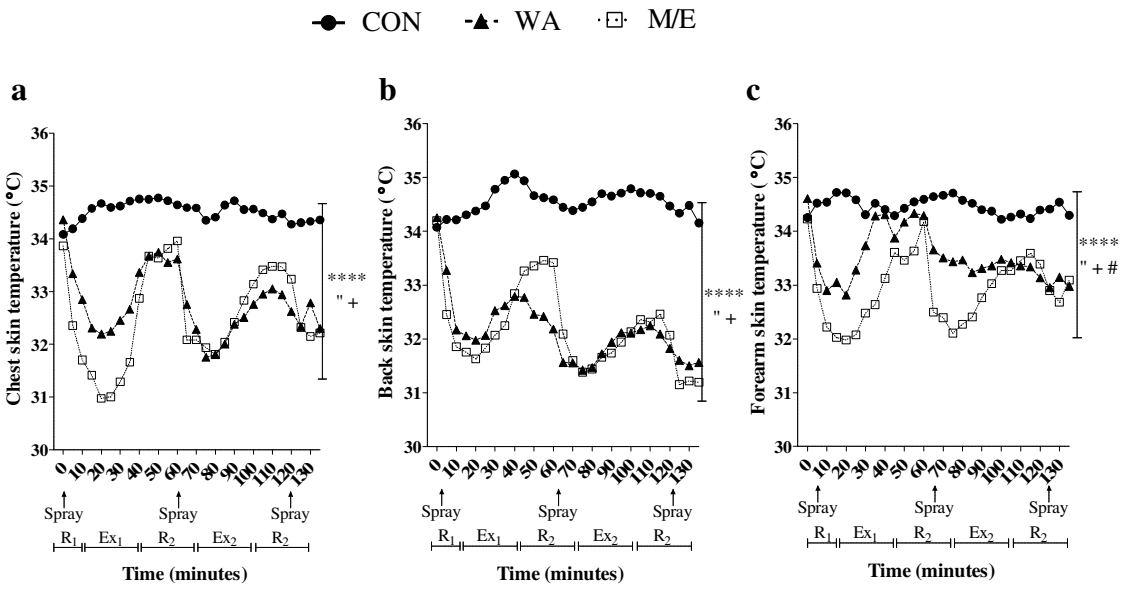


**Figure 10.** Median chest (a), back (b) and forearm (c) skin temperature during rest (R) and exercise (Ex) ($n = 6$). Significant difference (****$P < 0.0001$) by spray group (⌶). *Post-hoc* test: Significant difference between W and M/E (#, $P < 0.05$); between W and CON (**"**, $P < 0.001$); between M/E and CON (**+**, $P < 0.001$).

### *Thermal sensation*

Figure 11 shows mean whole body thermal sensation during exercise and rest, by group. A two-way ANOVA showed that mean TS differed over time ($P < 0.0001$) and by spray group ($P < 0.0001$), with an interaction ($P < 0.008$). *Post-hoc* testing showed that after baseline measures, TS remained lower (cooler) in M/E compared to W ($P < 0.01$) and CON ($P < 0.01$). The mean reduction in M/E (15$^{th}$ minute) was 6 TS units compared to CON, and 5 TS units compared to W.

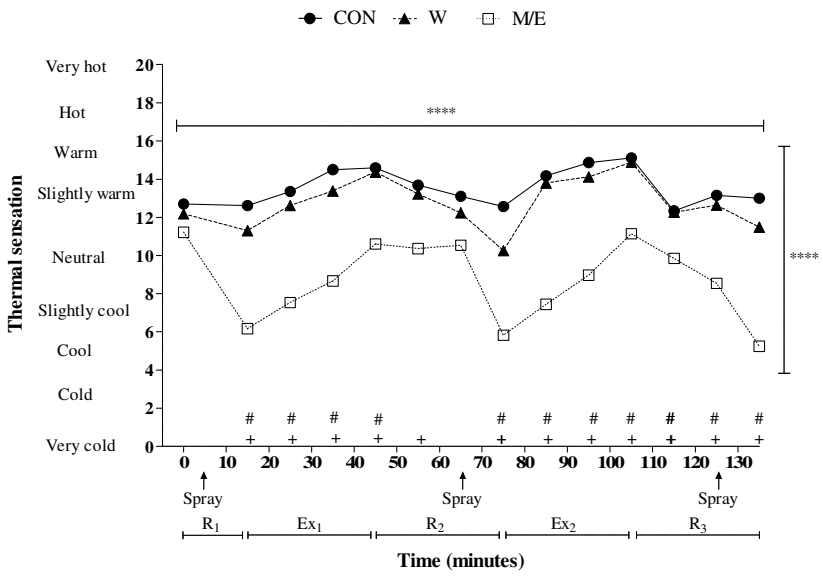


**Figure 11**. Mean whole body thermal sensation during rest and exercise, under conditions of no spraying, water spraying and 0.2 % menthol + 20 % ethanol spraying ($n$ = 6). Significant difference (****$P$ < 0.0001) by time (⊢⊣) and spray group (⌶). *Post-hoc* test: Significant difference between W and M/E (#, $P$ < 0.01); between M/E and CON (**+**, $P$ < 0.01).

***Thermal comfort***

Figure 12 shows mean whole body thermal comfort during exercise and rest, by group. A two-way ANOVA showed that TC differed over time ($P$ < 0.0001), and by spray group ($P$ = 0.034), with no interaction ($P$ > 0.05). The direction of effect could not be determined.

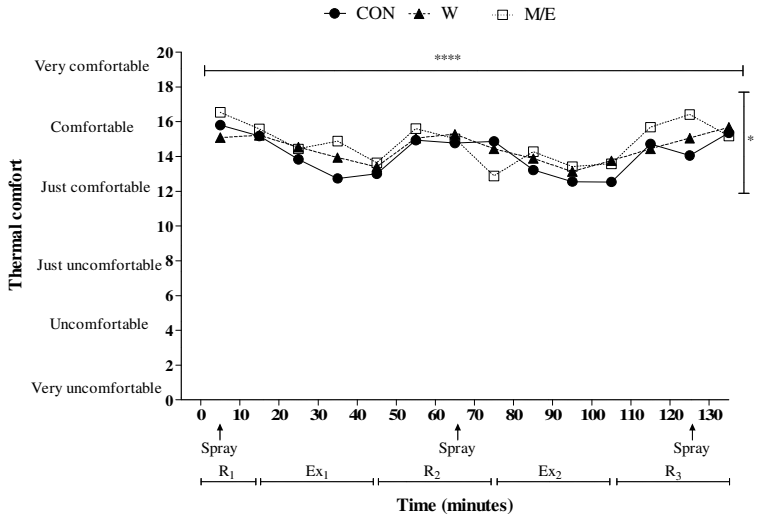


**Figure 12**. Mean whole body thermal comfort during rest and exercise, under conditions of no spraying, water spraying and 0.2 % menthol + 20 % ethanol spraying ($n$ = 6). Significant difference (* $P$ < 0.05; ****$P$ < 0.0001) by time (⊢⊣) and spray group (⌶).

## Discussion and Conclusions

A combined menthol/ethanol water-based spray was compared to water spraying and no spraying during exercise in warm, humid conditions to identify which intervention provided the greatest improvements in evaporative cooling and thermal perceptions.

The combination of stepping exercise and heat stress used in this study was sufficient to induce a cardiovascular and thermoregulatory challenge. Given this, the first noteworthy finding is that during exercise, M/E showed a greater $\Delta T_{re}$ and a lower skin temperature compared to CON and W; it is important to note that although statistically significant, the absolute difference in the $\Delta T_{re}$ between groups did not exceed 0.15 °C throughout testing. The biophysics of heat exchange dictates that a reduction in skin temperature should have increased the gradient of heat loss from the deep body to the periphery, and lowered $T_{re}$ in M/E compared to CON, but this was not observed because body temperature regulation is influenced by autonomic effector responses, in addition to passive biophysical processes. In this view, the inverse relationship between deep body and skin temperature in the M/E condition indicates that mean body temperature was stable during each exercise phase. However, this cannot be confirmed because mean body temperature was not calculated in this study, as this requires an accurate estimation of mean skin temperature, which could not be generated from only three sites (chest, back and forearm); nor could it be generated from the thermographic images because they only captured the upper torso.

However, other studies have shown a similar inverse relationship between deep body and skin temperature during exercise in response to skin cooling, and their findings support the notion that mean body temperature is the regulated variable. For example, Franks *et al.,* (1996) circulated either cool, warm, or no air over the bodies of participants walking on a treadmill (oxygen consumption; 1.2 L · $min^{-1}$) and showed that the cool air circulating condition had a strong inverse relationship between deep body and skin temperature, as did the warm circulating air condition but to a lesser extent; in this way the authors demonstrated that mean body temperature remained comparable across all conditions. The proportional input from deep body thermoreceptors (*i.e.* rectal temperature) in the study by Franks *et al.,* (1996) accounted for 93 % of the mean body temperature during exercise across the conditions. In the present study, it is not clear whether the proportional input from deep body thermoreceptors would change as a result of enhanced neuronal activation

of thermoreceptors in the skin arising from water and ethanol-mediated skin cooling, or menthol-mediated activation of the TRPM8 receptor. In any case, the inverse relationship between skin and deep body temperature in the M/E condition was likely driven by alterations in skin blood flow at rest (Savage & Brengelmann, 1996), and this could have been mediated by skin surface wetting, menthol activation of the TRPM8 receptor, and/or sweating during exercise; however, receptor function was not measured in this study, and neither was vasomotor or sudomotor function, so the underlying mechanisms driving the heat storage response require further investigation.

Savage and Brengelmann (1996) showed that at rest, a fall in skin temperature from water spraying reduces skin blood flow, which lowers heat loss and raises deep body temperature, resulting in an inverse relationship between the two. M/E spraying may have similarly lowered skin temperature at rest in the present study, which could have induced vasoconstriction and encouraged a rise in deep body temperature before exercise had begun. But the individual contributions of ethanol and water spraying, which may lower skin temperature to initiate vasoconstriction, and menthol, which probably initiates vasoconstriction without a reduction in skin temperature, both require clarification. During exercise, the increase in deep body temperature observed in the M/E condition is likely attributed to the action of menthol, rather than ethanol or water. Indeed, Kounalakis *et al.,* (2010) has proposed that menthol, although in higher doses than used in this study (*i.e.* 27.5 mg · 100 $cm^{-2}$ compared to 1.68 mg · 100 $cm^{-2}$), stimulates cold receptors which results in a withdrawal in sudomotor function and delays the onset of vasodilation during exercise, resulting in heat storage. This hypothesis cannot be confirmed at present, as there was no 'menthol-only' spray condition.

Given that ethanol can extract heat from the skin at a rate nearly twice that of water (Godts *et al.,* 2005) and the menthol/ethanol-based spray was applied repeatedly, its inability to sustain cooler skin temperatures compared to the water spray perhaps points to a methodological limitation. Although ethanol appeared to cool the skin more than water spraying or no spraying in the minutes immediately after its application, its influence appeared to wear-off, such that by the $30^{th}$ minute after spraying there was no visible difference in skin temperatures between M/E and W. Hence, it seems as though a period of 60 minutes between spraying was too long to maximise the evaporative cooling potential of ethanol. This suggests the optimum application frequency of a similar ethanol-based

solution would be every 30 minutes. However, this finding also suggests that water, which lowered the rate of rise in $T_{re}$ compared to CON and M/E, but is also less expensive and more abundant than ethanol, provides comparable evaporative cooling power in light work or exercise lasting longer than 30 minutes and, when sprayed on an hourly basis.

An important finding in this study was that participants felt significantly cooler (*i.e.* lower thermal sensation score) in M/E, compared to W and CON. Indeed, thermal sensation shifted by as much as six units on the TS scale, from feeling 'slightly warm' prior to spraying, to 'slightly cool' following menthol/ethanol spraying. That participants felt cooler in M/E compared to W *after* the ethanol had evaporated suggests that this effect was likely attributable to menthol, rather than ethanol. Although the notion that menthol enhances sensations of coolth when applied to the skin is not new (Watson *et al*., 1978; Green, 1992; Yosipovitch *et al.,* 1996; Wasner *et al*., 2004; Namer *et al*., 2005; Green & Schoen, 2007), this hypothesis cannot be confirmed at present, as there was no 'menthol only' spray condition.

In the present study, cool sensations in the M/E condition appeared to subside within 30 minutes, coinciding with the end of exercise and the evaporation of ethanol. It is interesting to note that although Yosipovitch *et al.,* (1996) applied a larger dose (625 mg · 100 $cm^{-2}$) of menthol to a smaller area (forearm) during rest, and the present study applied a smaller dose (1.68 mg · 100 $cm^{-2}$) to the entire upper body, both studies showed that the perceptual effects of menthol lasted 30 minutes. This raises a number of questions about the role of body surface area exposed and regional menthol sensitivity, but also about whether perceived exertion or elevations in body temperature following exercise may have diminished the perceptual influence of menthol. In any case, these findings raise the possibility of using a 0.2 % menthol-based water spray to enhance thermal perceptions in the heat.

It is interesting to note that thermal sensation improved (towards feeling cooler) similarly after the first, second and third applications of the menthol/ethanol solution. It was not possible to determine the relative contribution that each constituent (*i.e.* menthol or ethanol) played in this improvement, as they were held in solution together. In any case, this suggests that participants did not undergo any short-term habituation to the spray. This finding is in partial agreement with work by Cliff and Green (1994), who assessed cool sensations after repeatedly exposing participants to either 0.03 % or 0.3 % menthol (in the

oral cavity), separated by one to five minutes. The authors observed sensitization of cool sensations in some, and desensitization in others, suggesting there are large individual differences in menthol-induced sensations of coolth, at least in the oral cavity.

Notably, thermal comfort did not improve (*i.e.* move towards feeling more comfortable), with thermal sensation. Because exercise always followed spraying in this study, thermal comfort may not have improved as a result of increasing perception of effort, or perhaps an elevation in deep body temperature accompanying exercise. It is interesting to note that the ethanol-mediated reduction in skin temperatures, and the menthol-mediated improvement in thermal sensation were not enough to sway thermal comfort in either direction. Furthermore, it is difficult to isolate factors that may have influenced thermal comfort in this study. For example, Schlader *et al.,* (2009) highlighted the importance of skin temperature. But in the present study, cooling the skin caused no change in thermal comfort. Perhaps the skin was cooled too quickly, and when combined with the added perceptual cooling influence of menthol, contributed to a negative allesthesial response. Frank *et al.,* (1999) meanwhile, have suggested that both deep body and skin temperature contribute equally, and individually, to thermal comfort. With this view, the increase in $T_{re}$ observed during exercise would be expected to lower comfort, whilst the ethanol-mediated reduction in skin temperature should have enhanced it. The conflicting signals, when integrated in the somatosensory cortex, may have cancelled each other out, giving rise to the observation of no change in comfort. Similarly, Flouris and Cheung (2009) suggested that mean body temperature, combining deep body and skin temperature, likely drives thermal comfort: and although mean body temperature was not calculated in the present study, it probably would not have changed, as the menthol-mediated elevation in $T_{re}$ would have been balanced by the ethanol induced reduction in skin temperature. Given that thermal comfort also did not change in this study, this lends some support to the notion that mean body temperature was an important modulator of thermal comfort.

With regard to why thermal comfort did not improve with thermal sensation, anecdotally, some participants described feelings of irritation after menthol/ethanol spraying; so it is possible that the sensation of irritation prevented a clear improvement in thermal comfort. Further study is required to clarify the quality and intensity of irritation resulting from 0.2 % menthol spraying. It must be noted that up to 50 % of primary neurons that respond to cold and menthol also have the noxious heat receptor TRPV1 (McKemy *et al.,* 2002); and Green (2004) has suggested that some of the neurons that have TRPM8 receptors may also

project in the nociceptive (pain mediating) pathway rather than, or along with the cold pathway. Alternatively, an increase in skin wettedness has been shown to reduce comfort (Fukazawa & Havenith, 2009), and spraying the upper body of participants may have thereby prevented an overall improvement in comfort. Lastly, as previously alluded to, menthol/ethanol spraying may have induced sensations that were 'too cold' (*i.e.* negative allesthesia); indeed, a warm stimulus is not always considered comfortable, nor is a cold stimulus always uncomfortable, Cabanac's (1972) notion of alliesthesia supports this notion. That thermal comfort was not negatively altered following menthol/ethanol spraying raises the possibility of using a water-based menthol spray to improve thermal perceptions during exercise in the heat.

Given these findings, the null hypothesis that $T_{re}$ would not differ between groups is rejected in favour of the alternative hypothesis that $T_{re}$ is elevated during exercise following menthol/ethanol spraying. The null hypothesis that menthol/ethanol spraying has no influence over thermal sensation is rejected in favour of the alternative hypothesis that thermal sensation improves following menthol spraying. The null hypothesis that thermal comfort will not change following menthol/ethanol spraying is not rejected.

In summary, although 0.2 % menthol + 20 % ethanol spraying induced an elevation in deep body temperature compared to water spraying, the absolute difference was never greater than 0.15 °C. Furthermore, water spraying provided comparable evaporative cooling to ethanol/menthol/water spraying, and it is more cost effective and convenient to use, especially in the long term (when sprayed at intervals greater than 30 minutes). It seems menthol/ethanol solution spraying results in cooler sensations than water spraying or no spraying, but does not influence thermal comfort, possibly due to thermoregulatory and/or perceptual responses. These collective responses are thought to be due to the action of menthol in the 0.2 % menthol + 20 % ethanol solution spray, but further testing is required to confirm this hypothesis. These findings raise the possibility of using a water-based 0.2 % menthol spray as a cost effective cooling intervention to enhance evaporative heat loss and thermal sensations compared to no spraying, during exercise in warm, humid conditions. This hypothesis was tested in Study two.

# Chapter 5

# Study 2: The influence of 0.2 % menthol solution spraying on deep body temperature and perception during exercise in warm, humid conditions

## Introduction

Study one showed that repeatedly applying a 20 % ethanol + 0.2 % menthol solution improved evaporative cooling compared to water spraying and no spraying in the short term, but it was comparable to water spraying beyond 30 minutes. Both sprays enhanced evaporation compared to no spraying. Menthol/ethanol/water spraying increased heat storage compared to water spraying, but also caused the coolest sensations compared to all conditions; it neither improved, nor impaired thermal comfort, possibly due to an interaction with thermoregulatory and/or perceptual responses. Given that water spraying cooled the skin comparably to the ethanol/menthol/water spray beyond 30 minutes, without inducing any heat storage response, and was more cost effective, it was recommended to UK Sport over the menthol/ethanol spray. But the perceptual cooling power of the menthol/ethanol spray was intriguing and hypothesised to be due to the action of menthol; raising the possibility of using it as a perceptual cooling intervention during rest or exercise in the heat with some capacity to enhance evaporative heat loss, but little is known of menthol's influence on perception and thermoregulation during rest and exercise.

Two peer reviewed studies have assessed the influence of menthol on thermoregulation (Kounalakis *et al.,* 2010) and thermal perceptions (Schlader *et al.,* 2011a) during exercise. Kounalakis *et al.,* showed that participants' deep body temperature increase more quickly after menthol was spread over the whole body; also, the time of sweating onset was delayed, along with the change in rectal temperature required to initiate sweating. The difference in forearm and finger tip temperature, taken as an index of vasoconstriction (House & Tipton, 2002), was also greater, indicating a lower skin blood flow and a delay in the onset of vasodilation for the first 10 minutes of exercise. Kounalakis *et al.,* used a dose of menthol that was approximately 15 times larger than the dose used by most commercial companies (*i.e.* 1.6 mg vs. 27.5 mg · 100 $cm^{-2}$), so it is not clear whether the heat storage observed by those authors applies to all menthol doses; furthermore, the authors did not report any perceptual responses, so the influence of menthol on the more global sensations of thermal comfort, irritation and perceived exertion await clarification.

In the other study, Schlader *et al.,* (2011a) evaluated the independent roles of thermal perception and skin temperature in guiding behaviour by allowing participants to exercise at a fixed rating of perceived exertion, while undergoing either face cooling, face warming, or simulated face cooling (8 % menthol gel, 500 mg · 100 $cm^{-2}$), or warming (0.025 % capsaicin cream), or during a Control condition where their face was left alone. In this design, both face cooling and menthol improved thermal sensation and comfort, both of which lead to higher power outputs and longer exercise duration. But because the exercise protocol was fixed to a predetermined level of perceived exertion, rather than to a percentage of maximal power output, work-rate (and metabolic heat production) differed during each test. It is therefore difficult to separate the perceptual influence of menthol from the perceptions arising from different work-rates and metabolic heat productions. Further research is required to assess the perceptual influence of menthol during fixed rate exercise, when metabolic heat production is controlled. Also, the authors used a dose that was approximately 300 times greater than the dose used by most commercial companies (*i.e.* 1.6 mg compared to 500 mg · 100 $cm^{-2}$), therefore it is not clear whether the findings described in this study extend to all menthol doses.

**Aims and Hypotheses**

The primary aim of this study was to characterise the influence of menthol on heat storage and thermal perceptions in heat stressed humans. To this end, a low dose 0.2 % menthol solution spray or a water spray (control) was repeatedly sprayed onto breathable long sleeve shirts and worn during rest, mild and fixed high intensity exercise in warm, humid conditions (30 °C, 70 % rh).

***Null hypothesis***

1. There will be no difference in thermal comfort or ratings of perceived exertion between 0.2 % menthol solution spraying and water spraying during rest and exercise.

***Alternative hypotheses***

1) During exercise, the change in $T_{re}$ will be greater following 0.2 % menthol solution spraying compared to water spraying.

2) 0.2 % menthol solution spraying will improve thermal sensation and increase reports of irritation more than water spraying during rest and exercise.

## Methods

### *Participants*

Eight participants visited the environmental laboratory on three occasions. Mean (SD) participant age, height and mass were: 23 (2.26) years, 180.9 (8.2) cm and 77.7 (9.6) kg.

### *Procedure*

On their first day, participants were asked to complete a $PO_{peak}$ test. Mean (SD) $PO_{peak}$ was 353.0 (59.1) W. On the second and third days, which were separated by one day, they completed two 115 minute cycling tests in 30 °C, 70 % rh. Each participant underwent two upper body spray conditions; 0.2 % menthol solution ($M_{0.2\%}$) or water (CON) spraying. Participants wore long sleeve breathable shirts, footwear, socks and shorts during each test.

As the main aim of this study was to assess the influence of 0.2 % menthol solution spraying during rest and exercise undertaken at different intensities, cycle ergometry was chosen over stepping exercise (Study one) in an effort to more precisely monitor and control work-rate throughout the trial. Participants entered the environmental chamber at time zero and sat on the cycle ergometer (Monark) for five minutes while they became accustomed to the environmental conditions. In an effort to reflect a realistic sporting scenario, they undertook 10 minutes of warm-up exercise at 35 % of their peak power (mean [SD] $PO_{35\%}$; 123.5 [20.5] W). After warming up, they sat resting for 15 minutes. At this point they were sprayed with either the 0.2 % menthol solution or water. To clarify menthol's influence on resting participants, they remained seated for an additional 15 minutes after spraying. After this 15 minute period, participants were sprayed for a second time and immediately began cycling at $PO_{45\%}$ (mean [SD]; 158.9 [26.6] W) in order to clarify the influence of 0.2 % menthol solution spraying on moderately exercising participants. After 45 minutes of exercise at $PO_{45\%}$, participants stopped cycling, were sprayed for a third time, and began cycling at $PO_{70\%}$ (mean [SD]; 247.1 [47.4] W) in order to assess the influence of 0.2 % menthol solution spraying on participants exercising at a high intensity. Participants cycled at $PO_{70\%}$ until 15 minutes had elapsed, or until they could no longer continue. The experimental timeline for Study two is shown below.

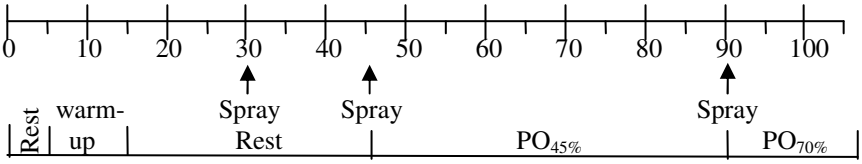


**Figure 13.** Study two experimental timeline.

Participants were asked to report TC, TS, RPE, and irritation, and HR was recorded at minutes 5, 10, 20, 35, 40, 50, 60, 70, and 80. $T_{re}$ was measured and skin temperature recorded at the chest, forearm, thigh and calf by the minute. $\overline{T}_{msk}$ (Ramanathan, 1964) and $\overline{T}_b$ (Burton, 1935) were calculated.

***Analyses***

A two-way repeated measure ANOVA, by spray group and time (and interaction) was used to assess the significance of parametric data. Non-parametric data were analysed using the Wilcoxon matched-pairs sign rank test, with a correction for multiple comparisons and reported as median (range) scores. The alpha level was set at 0.05, unless otherwise specified. All analyses were performed on the data up to the 90$^{th}$ minute, as participants began dropping out afterwards. The data from one participant were removed from $T_{re}$ analysis (and $\overline{T}_b$) due to the rectal thermistor slipping out, leaving a sample size of seven.

## Results

***Environmental conditions***

Environmental temperature and rh did not significantly differ by spray group ($P > 0.05$). Mean (SD) dry air, globe and wet bulb temperatures were 30.8 (0.3) °C; 30.8 (0.5) °C; and 26.4 (0.4) °C respectively. Mean (SD) relative humidity was 67.6 (2.0) %.

***Measures of work-rate***

Overall mean (SD) heart rate remained stable at 80 (11.6) beats · min$^{-1}$ during both resting phases, then rose to 126 (12.4) beats · min$^{-1}$ during the warm-up, and approached 153 (13.5) beats · min$^{-1}$ during exercise at $PO_{45\%.}$ A two-way ANOVA showed a difference in HR over time ($P < 0.0001$), but not by spray group ($P > 0.05$), with no interaction ($P > 0.05$).

During the warm-up, RPE was described as ‘very light’ to ‘light’. At the start of exercise at $PO_{45\ \%}$, participants in both groups perceived their effort to be ‘light’ to ‘somewhat heavy’, and ‘somewhat heavy’ to ‘heavy’ by the end of exercise. A non-parametric Wilcoxon test compared starting and ending RPE, and the change in RPE over this time by spray group. Without correcting for multiple comparisons, spray groups did not differ in the starting ($P = 0.070$) or ending ($P = 0.161$) RPE, or the change in RPE over this time ($P = 0.712$). The median (range) starting and ending RPE during $PO_{45\ \%}$ were: 13 (11 to 14) and 15 (14 to 17) for CON and 12 (8 to 13) and 14 (12 to 18) for $M_{0.2\ \%}$ respectively.

All participants completed 45 minutes of exercise at $PO_{45\ \%}$. At the 90th minute, they were sprayed and increased the intensity of exercise to $PO_{70\ \%}$, and were told to exercise until exhaustion. There was no difference ($P > 0.05$) in the time to exhaustion between groups, which was (mean [SD]) 96.8 (4.6) minutes in $M_{0.2\ \%}$ and 96.7 (3.8) minutes in CON; no one was able to complete 15 minutes of exercise at $PO_{70\ \%}$.

***Rectal temperature***

Figure 14 shows mean $T_{re}$ during exercise and rest, by spray group. A two-way ANOVA showed a difference by time ($P < 0.0001$) and spray group ($P < 0.0001$) with no interaction ($P > 0.05$). *Post-hoc* testing did not detect the direction of effect ($P > 0.05$). A t-test comparing the $\Delta T_{re}$ during exercise from minute 15 to 90 showed a greater (mean [SD]) $\Delta T_{re}$ in $M_{0.2\%}$ (0.9 [0.3] °C) compared to CON (0.8 [0.2] °C) ($P = 0.029$).

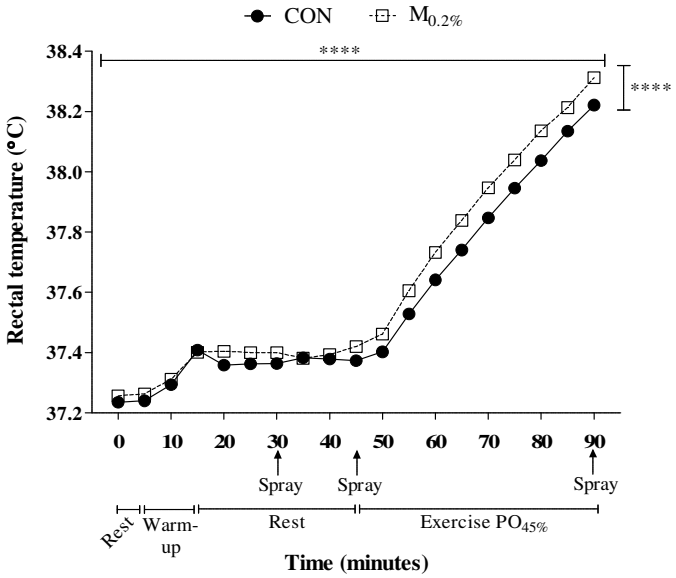


**Figure 14**. Mean rectal temperature during rest and exercise, by spray group ($n = 7$). Significant difference (****$P < 0.0001$) by time (⊢⊣) and by spray group (⌶).

***Mean skin temperature***

Figure 15 shows $\overline{T}_{msk}$ during exercise and rest, by group. A two-way ANOVA showed a difference by time ($P < 0.0001$) and spray group ($P = 0.018$), with no interaction ($P > 0.05$). *Post-hoc* testing could not detect the direction of effect ($P > 0.05$).

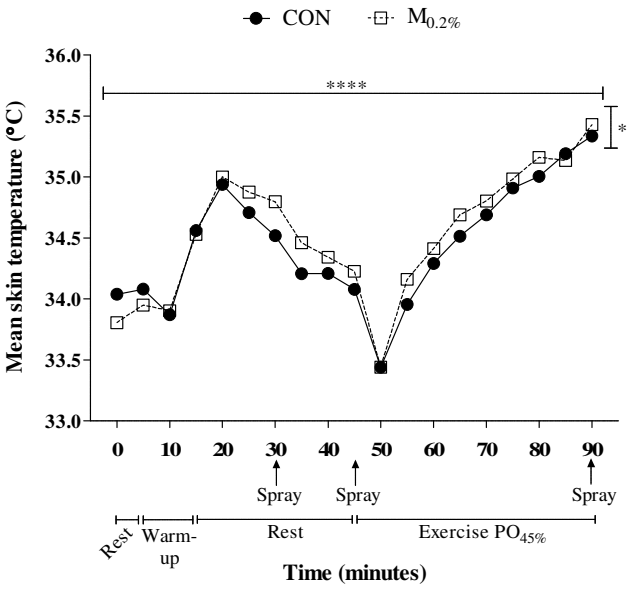


**Figure 15.** Mean skin temperature during exercise and rest, by spray group ($n$ = 8). Significant difference (*$P$ < 0.05; ****$P$ < 0.0001) by time (⊢⊣) and by spray group (⌶).

***Mean body temperature***

Figure 16 shows $\overline{T}_b$ during rest and exercise, by group. A two-way ANOVA showed a difference by time ($P$ < 0.0001) and spray group ($P$ = 0.0006), with no interaction ($P$ > 0.05). *Post-hoc* testing could not detect the direction of effect ($P$ > 0.05).

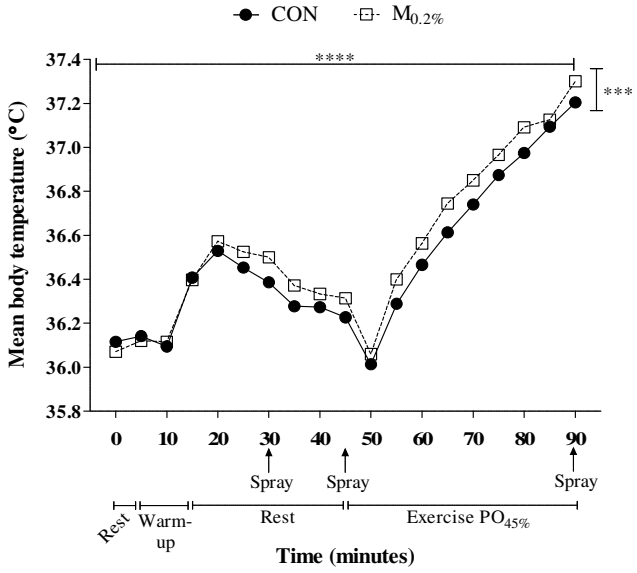


**Figure 16.** Mean body temperature during exercise and rest, by spray group ($n$ = 7). Significant difference (***$P$ < 0.001; ****$P$ < 0.0001) by time (⊢⊣) and spray group (⌶).

***Thermal comfort***

Figure 17 shows thermal comfort during rest and exercise by spray group. A two-way ANOVA showed that TC did not differ over time ($P > 0.05$), but did differ by spray group ($P = 0.0065$), with an interaction ($P < 0.0001$). *Post-hoc* testing showed TC remained lower (more uncomfortable) during rest at the 35$^{th}$ and 40$^{th}$ minutes, following 0.2 % menthol spraying ($P < 0.01$). The mean difference between $M_{0.2\%}$ and CON was 4 TC units, equating to a shift from feeling 'comfortable' to 'just comfortable'.

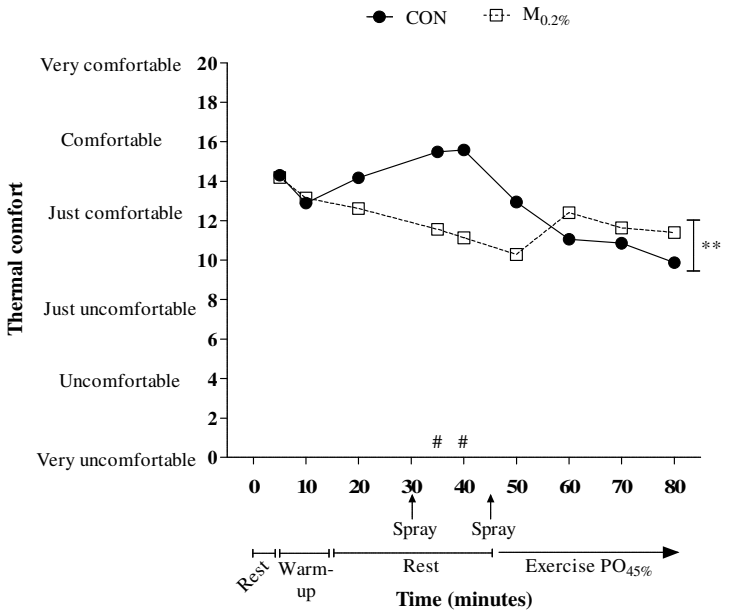


**Figure 17.** Mean upper body thermal comfort during exercise and rest, by spray group ($n$ = 8). Significant difference (**$P < 0.01$) by spray group (⌶). *Post-hoc* test: Significant difference between CON and $M_{0.2\%}$ (#, $P < 0.01$).

***Thermal sensation***

Figure 18 shows thermal sensation during exercise and rest, by group. A two-way ANOVA showed that TS differed over time ($P < 0.0001$) and between spray groups ($P < 0.0001$), with an interaction ($P = 0.0189$). *Post-hoc* testing showed TS remained lower (cooler sensations) between the 35$^{th}$ and 60$^{th}$ minutes in $M_{0.2\ \%}$ ($P < 0.05$). The mean difference during this period equated to 4.5 units on the TS scale.

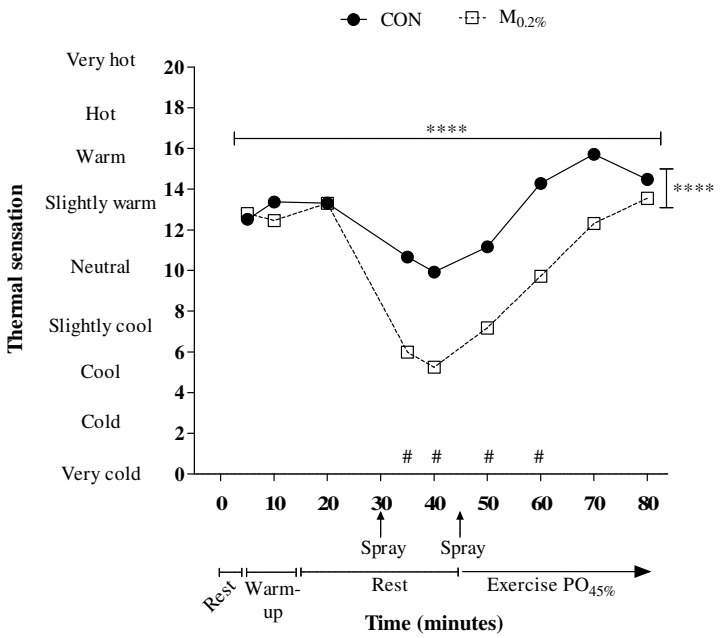


**Figure 18**. Mean upper body thermal sensation during exercise and rest, by spray group ($n$ = 8). Significant difference (****$P < 0.0001$) by time (⊢⊣) and by spray group (⌶). *Post-hoc* test: Significant difference between CON and $M_{0.2\%}$ (#, $P < 0.05$).

***Irritation***

Participants were asked to report their irritation on nine occasions during testing; at minutes 5, 10, 20, 35, 40, 50, 60, 70, and 80. Figure 19 shows the number of participants that reported some form of irritation during testing. No participants noted any irritation with water spraying, so the data displayed in Figure 19 only represent participants in $M_{0.2\%}$. During post spraying resting measurements, seven out of eight participants noted irritation. During the early phase of exercise, six reported irritation, but as exercise continued fewer noted any irritation, and by the 80th minute, no one reported any sensations of irritation.

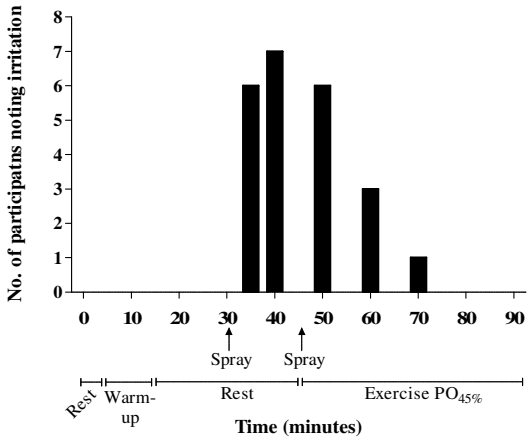


**Figure 19**. Number of participants noting irritation after menthol spraying, by time.

Table 1 shows the quality of the irritation experienced by the participants during testing. All participants noted some form of irritation. Reports of tingling were most common, followed by burning, skin abrasions, prickling, and numbness.

**Table 1.** Description of irritation, by participant and time, after menthol spraying

| | Experimental time (minute) | | | | |
|---|---|---|---|---|---|
| **Participant** | **35$^{th}$** | **40$^{th}$** | **50$^{th}$** | **60$^{th}$** | **70$^{th}$** |
| **S1** | Tingling | Tingling | Tingling | | |
| **S2** | Tingling | | | | |
| **S3** | Burning | Burning | | | |
| **S4** | Tingling | Burning | Burning | | |
| **S5** | | Tingling | Tingling | | |
| **S6** | | Burning, skin abrasions | Burning, skin abrasions | Burning, skin abrasions | |
| **S7** | Skin abrasions | Skin abrasions | Skin abrasions | Skin abrasions | |
| **S8** | Numbness, tingling | Stinging, pricking, burning | Tingling, prickling | Tingling, prickling | Prickling |

## Discussion and Conclusions

A 0.2 % menthol solution spray was compared to a water spray so as to characterise the influence of the menthol compound on heat storage and thermal perceptions during rest and exercise in warm, humid conditions. It is important to note that water spraying was chosen as a Control condition rather than a no-spray condition because Study one demonstrated that water spraying enhanced evaporative heat loss comparably to ethanol, especially after 30 minutes, and more than the no-spraying condition.

The combination of cycle ergometry and heat stress used in this study was sufficient to induce a cardiovascular and thermoregulatory challenge, particularly during exercise at $PO_{45\ \%}$; however, participants could not complete more than seven minutes of exercise on average at $PO_{70\ \%}$ in either group (range; two to 11 minutes), perhaps (anecdotally) due to leg fatigue, so data beyond the 90$^{th}$ minute have been omitted.

Most notably, the $\Delta T_{re}$ from the 15$^{th}$ to the 90$^{th}$ minute was significantly greater in $M_{0.2\ \%}$ compared to CON, by 0.11 °C. This finding confirms that 0.2 % menthol, in isolation of 20 % ethanol, causes heat storage, and supports the work by Kounalakis *et al.,* (2010). Although the underlying mechanisms mediating this response are not clear, a reduction in skin blood flow and a delay in the onset and magnitude of sweating have been suggested (Kounalakis *et al.,* 2010). Skin temperature can be used as an indirect measure of both

effector mechanisms, whereby vasoconstriction reduces skin temperature whilst a delay in the onset of sweating, or a lower magnitude, increases it. $\overline{T}_{msk}$ was observed to differ by group, but *post-hoc* testing could not detect the direction of effect. Visually, it appeared as though skin temperature was higher in $M_{0.2\ \%}$ (*i.e.* 0.1 °C at minute 25, Figure 15) even prior to spraying and this difference appeared to persist throughout the remainder of the test. It should be noted that under the conditions of this study it is difficult to separate any reductions in skin temperature resulting from vasoconstriction or sweat evaporation, from those resulting from water evaporation; hence, further research is required to clarify the underlying effector mechanisms driving heat storage following 0.2 % menthol solution spraying through direct observation of both vasomotor and sudomotor function.

Immediately after the warm-up period, both rectal and mean skin temperatures appeared to fall more quickly when participants underwent control spraying compared to 0.2 % menthol spraying. This cooling is visible in the figures for $T_{re}$, $\overline{T}_{msk}$ and $\overline{T}_{b}$, (Figures 14 to 16) from the $15^{th}$ to the $30^{th}$ minute. This difference is not likely to be due to the environmental conditions, as they did not differ by spray condition, nor might it have been due to circadian variations, as participants completed each experiment at a similar time of day. Although the seating of all skin temperature thermistors were regularly checked during testing, and the individual data were assessed post testing, it is possible that some thermistors may have become loose between the skin surface and the adhesive tape and rotated away from the skin, or perhaps fell off momentarily during testing, hence underestimating $\overline{T}_{msk}$ in the water spraying condition. It is also possible that the difference in temperatures, which approached 0.2 °C for $\overline{T}_{msk}$ and 0.1 °C for $\overline{T}_{b}$ between conditions, represents normal variation in these responses. The estimation of $\overline{T}_{msk}$ might be improved in future studies by adding more measurement sites. Although the four site formula developed by Ramanathan (1964) was expected to accurately estimate $\overline{T}_{msk}$ in the present study during exercise in the heat, primarily due to the increase in skin temperature uniformity following peripheral vasodilation, these four sites may not have reliably estimated $\overline{T}_{msk}$ under the conditions of this study. For example, although exercising in the heat will result in a more uniform skin temperature, skin wetting with water may result in vasoconstriction and less uniformity, as might menthol; hence more sites, both sprayed and unsprayed, may be required to estimate $\overline{T}_{msk}$ more precisely in future studies

A key finding in this study was that thermal sensation was significantly altered following menthol solution spraying, compared to water spraying alone. Furthermore, this difference occurred despite a lower mean skin temperature in the Control condition. This confirms that the 0.2 % menthol solution, in isolation of 20 % ethanol, elicits cool sensations, and supports the assertion that menthol enhances sensations of coolth when applied to the skin (Watson *et al.,* 1978; Green, 1992; Yosipovitch *et al.,* 1996; Wasner *et al.,* 2004; Namer *et al.,* 2005; Green & Schoen, 2007). In this study, menthol exerted its influence only after it was absorbed across the fabric barrier and came into contact with the skin. This process occurred very quickly after spraying, as menthol began to exert a clear perceptual influence on thermal sensation within five minutes of application (by the 35$^{th}$ minute), and throughout the remainder of the resting period. The change in perception was also noted after the second spraying, during exercise. The sensation of coolth lasted for approximately 30 minutes, after which time a visible, but non-significant difference was observed until the 80$^{th}$ minute. Because participants were sprayed repeatedly with either spray, it is difficult to determine the influence of a single application of 0.2 % menthol on perception, both in the duration and intensity of perceived cooling. Indeed, a single spraying left 2.1 mg · 100 cm$^{-2}$ of menthol on the skin of the upper body, and each subsequent spraying left the same amount. As menthol is an alcohol, it will vaporise, but the rate that 0.2 % vaporises when bound in a solution with 3 % surfactant is not known. Martin *et al.,* (2004) has shown that very low doses of menthol (comparable to this study) will cross into the blood stream, which suggests that the rate of vaporisation is slow; however, the amount of menthol absorbed into the blood stream was also very small in the study by Martin *et al.,* (2004). Therefore, it is likely that some menthol was present in the fabric of the shirt, or on the participant's skin after experimentation. To remove excess menthol from the skin and shirt before subsequent testing was carried out, showering and machine washing were undertaken.

Similar to Study one, thermal comfort did not change with thermal sensation. Indeed, participants felt significantly greater thermal discomfort following menthol spraying whilst they rested after the warm-up period, particularly at minutes 35 and 40. It is possible that the significant, although slight, elevations in $T_{re}$ and $\overline{T}_{msk,}$ (and $\overline{T}_{b}$) reduced thermal comfort at this early stage, although *post-hoc* testing of the thermometry data does not support this notion. It is interesting to note that at the 35$^{th}$ and 40$^{th}$ minutes, the strongest feelings of discomfort coincided with the coolest thermal sensations. This finding perhaps supports

Cabanac's notion of allesthesia (1972), whereby the cooling action of menthol may have been too cool to improve thermal comfort. Alternatively, the number of participants noting irritation was also greatest at the 35$^{th}$ and 40$^{th}$ minutes, suggesting a causal relationship between irritation and thermal comfort. Further research is required to quantify the intensity of irritation, in addition to the quality (*i.e.* burning, tingling), and the number of reports, so as to better understand the relationship between thermal comfort and irritation.

In this study, a significant interaction was observed between time and spray condition for thermal comfort. Specifically, when participants underwent water spraying during rest, their comfort improved until the onset of exercise, after which time their perception of comfort appeared to decline (they felt greater thermal discomfort). The opposite pattern was observed with menthol spraying, whereby thermal comfort lowered during rest (felt greater discomfort), and then rose during exercise (felt less thermal discomfort). The menthol-mediated interaction between TC, rest and exercise requires clarification; indeed, numerous possibilities can be proposed to explain it. For example, menthol spraying induced a significant elevation in $T_{re}$, so it is possible that elevations in deep body temperature accompanying exercise and ratings of perceived exertion may have "drowned-out" sensations of irritation, allowing for an improvement to TC during exercise with menthol spraying. It is also possible that as participants stored heat, the negative allesthesial response turned positive, and sensations that were once perceived as "too cool", eventually proved sufficient to improve thermal comfort. Lastly, the influence of menthol on cool sensations and irritation may have simply "worn off", possibly due to receptor adaptation or menthol clearance from the skin to the blood, thereby allowing for an improvement in thermal comfort. On this note, at the 60$^{th}$ minute, when a clear improvement in TC was noted following menthol spraying, the number of reports of irritation had halved, but participants still felt significantly cooler, implying that irritation primarily influenced TC at this point. That sensations of irritation can be modulated separately from sensations of coolth is intriguing. On this note, it has been suggested that cool sensation varies directly with menthol dose, as does irritation, but cool sensation seems to vary less than irritation (Cliff & Green, 1994). Therefore, by reducing the dose of menthol in a given spray, it may be possible to minimize sensations of irritation and improve thermal comfort. Further research is required to clarify the dose/response of menthol, from both a perceptual and thermoregulatory perspective.

Given these findings, the null hypotheses that there would be no difference in $T_{re}$, thermal sensation and sensations of irritation between spray conditions can all be rejected in favour of the alternative hypotheses that 0.2 % menthol spraying mediates heat storage, brings about cooler sensations and increases sensations of irritation. The null hypothesis that thermal comfort would not change between spray conditions is rejected in favour of the alternative hypothesis that thermal comfort is reduced following menthol spraying at rest, but does not change during exercise compared to water spraying. Lastly, the null hypothesis that RPE would not change is not rejected.

In summary, although 0.2 % menthol solution spraying induced an elevation in deep body temperature during exercise, the absolute average difference was never greater than 0.1 °C. This is attributed to the action of menthol, but the underlying mechanisms driving this heat storage require further clarification. Further, although significant, the practical implications of this elevation in $T_{re}$ are not clear. It seems 0.2 % menthol solution spraying results in cooler sensations than water spraying, and impairs thermal comfort at rest, but not during exercise, possibly due to an interaction with irritation. Lowering the dose of menthol may reduce sensations of irritation, and preserve sensations of coolth. This hypothesis was tested in Study three.

# Chapter 6

# Study 3: The influence of 0.05 % and 0.2 % menthol spraying on thermoregulation and perception during exercise in warm, humid conditions

## Introduction

Study two showed that a water-based solution containing 0.2 % menthol enhanced sensations of coolth, and reduced thermal comfort during rest with no change during exercise, possibly due to irritation, compared to a water-only spray. From a perceptual perspective, if these sensations of irritation could be minimized and cool sensations preserved, menthol could serve as a perceptual cooling intervention during exercise in the heat. However, the heat storage response, which also appears to be mediated by menthol, requires further study before any such intervention is recommend.

It has been suggested that cool sensation varies directly with menthol dose, as does irritation, but cool sensation seems to vary less than irritation (Cliff & Green, 1994). Therefore, by reducing the dose of menthol in a given solution, it may be possible to minimise sensations of irritation, whilst maintaining cool sensations. Cliff and Green (1994) have noted that doses of 0.03 % menthol preserve cool sensations but reduce the irritation caused by a 0.3 % menthol solution, at least in the oral cavity. Given the paucity of research in this area, pilot testing a range of menthol doses was necessary to identify a low-end that still induces cool sensations. To this end, four different doses were assessed for their perceptual cooling effect; 0.2 %, 0.15 %, 0.1 % and 0.05 %. Eight individuals volunteered to take part in this informal test, six female, two male (sample of convenience). Each dose was placed on a different section of the arm of each volunteer (left and right bicep and forearms). Each volunteer was blinded to the dose of menthol at each location, while the investigator was aware. The volunteers were instructed to rank the perceived coolness of each exposed region. Results indicated that the 0.2 % menthol solution produced the strongest cooling effects, while the 0.05 % solution the least. Most volunteers found it difficult to differentiate between the middle two doses. Given that the 0.05 % solution was able to induced noticeably cool sensations, it was chosen as a comparator to the 0.2 % menthol spray, which produced the coolest sensations. Hence, a 0.05 % and 0.2 % menthol solution spray were compared to a water spray in this study.

Study two also demonstrated that 0.2 % menthol solution spraying induced an elevation in deep body temperature during exercise; however, the absolute difference was never greater than 0.1 °C. Although this was attributed to the action of menthol, the underlying mechanisms mediating this response were not clear; however, a reduction in skin blood flow and a delay in the onset of sweating have been implicated (Kounalakis *et al.,* 2010).

As early as 1924, scientists recognised that menthol is not only an inert cooling compound or placebo, indeed an anonymous author, writing about the cooling effect of menthol in the journal *California and Western Medicine*, even speculated that peripheral vasoconstriction probably followed from stimulation of higher centres in the brain, particularly the vasomotor centre (Anonymous, 1924). Unfortunately, there is limited research to support this. For example, Yosipovitch *et al.,* (1996) applied 10 % (620 mg · 100 $cm^{-2}$) menthol to the forearm and showed no difference in skin blood flow after application. Alternatively, an increase in skin blood flow was observed following application of 40 % menthol (3,200 mg · 100 $cm^{-2}$) to the forearm (Wasner *et al.,* 2004). Namer *et al*., (2005) also showed an increase in skin blood flow following application of 40 % menthol (640 mg · 100 $cm^{-2}$) to the forearm, possibly owing to an inflammatory response. Similarly, Johnson *et al.,* (2009) also showed an increase in forearm skin blood flow following application of a 3 % menthol solution to the forearm (containing 25 % ethanol, volume unspecified). Alternatively, Olive *et al.,* (2010), observed a significant reduction in forearm vascular conductance after applying either 3.5 % menthol (17.5 mg · 100 $cm^{-2}$) or ice to the forearm. Unfortunately, this last study did not benefit from an adequate Control condition, so the cooling influence of the gel that suspended menthol in solution could not be determined.

In one of the few studies to assess the influence of menthol on whole body temperature regulation during exercise, Kounalakis *et al*., (2010) showed that participants' deep body temperature increased more quickly after menthol spreading; furthermore, the time of sweating onset was delayed, along with the change in rectal temperature required to initiate sweating. The difference in forearm and finger-tip temperature, taken as an index of vasoconstriction (House & Tipton, 2002), was also greater, indicating a lower skin blood flow and a delay in the onset of vasodilation for the first 10 minutes of exercise. The authors proposed that menthol stimulated cold receptors which in turn deactivated the sweating response by reciprocal cross inhibition (Sherrington, 1906; Bligh, 1998; Kounalakis *et al.,* 2010; Mekjavic & Eiken, 2006). Unfortunately, it is not possible to

confirm this theory, as the pathways mediating the increased sympathetic cholinergic outflow to sweating glands is at present unknown (Morrison & Nakamura, 2011), and numerous non-thermal factors may influence it (Mekjavic & Eiken, 2006), such as muscular activity (Yanagimoto *et al.,* 2003) or mental stress (Machado-Moreira & Taylor, 2011). Also, neither skin, nor deep body temperature responses were reported, so it was not possible to determine whether an inverse relationship was demonstrated between deep body and skin temperature, and whether mean body temperature was stable (regulated) across groups. Also, the authors did not measure skin blood flow directly, and used a much higher dose of menthol than is used in the present study (*i.e.* 27.5 mg compared to 2.1 mg or 0.52 mg per 100 $cm^{-2}$). Thus, further clarification of the underlying mechanisms driving heat storage is required.

## Aims and Hypotheses

The primary aim of this study was to explore whether lowering the dose of menthol from 0.2 % to 0.05 % could minimise perceptions of irritation and improve thermal comfort, whilst maintaining cool sensations. The secondary aim was to characterise the underlying mechanisms of the heat storage response observed following 0.2 % menthol spraying.

### *Null hypotheses*

1. There will be no difference in physiological or perceptual responses between the two menthol solutions during rest or exercise.
2. During exercise, RPE and TC will not differ between any of the spray conditions.

### *Alternative hypotheses*

1. Both menthol solutions will improve thermal sensation and increase irritation during rest and exercise, and reduce thermal comfort at rest compared to water spraying.
2. Both menthol solutions will cause a heat storage response compared to water spraying.

## Methods

### *Participants*

Twelve participants volunteered for this study; their mean (SD) characteristics were: age 22 (2.9) years; weight 75.7 (8.7) kg and height 179.1 (6.6) cm. Mean (SD) $\dot{V}O_{2peak}$, $PO_{peak}$ and $PO_{45\ \%}$ were: 47.4 (6.2) $mL{\cdot}kg^{-1}{\cdot}min^{-1}$; 349.9 (41.8) W and 157.4 (18.8) W respectively.

***Procedure***

Participants first completed one $PO_{peak}$ test, and then three exercise tests in an air temperature of 30 °C and 70 % rh after having been sprayed with 100 mL of either: 0.05 % menthol solution ($M_{0.05\ \%}$), 0.2 % menthol solution ($M_{0.2\ \%}$), or a Control spray (CON; water only). The three exercise tests were completed in a balanced order. During each test participants entered the environmental chamber and remained seated on a cycle ergometer for 20 minutes to achieve thermal balance, after which time they were sprayed with water (CON) or a solution containing either 0.2 % or 0.05 % menthol. Participants remained seated on the cycle ergometer for an additional 15 minutes after spraying. They then began to exercise at $PO_{45\ \%}$ for 45 minutes, after which the test was terminated.

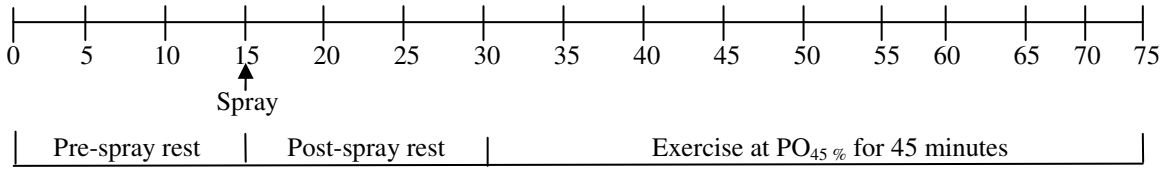


**Figure 20.** Study three experimental timeline.

Given that the main aim of this study was to clarify the dose-response characteristics of menthol, a single, rather than multiple applications of each spray was chosen to simplify the comparison. Testing included a 15 minute post spraying rest period, followed by moderate exercise, in order to assess menthol's influence in both a resting and active physiological state. Moderate intensity exercise ($PO_{45\ \%}$) was chosen over higher intensity exercise in the hope that participants would achieve a thermoregulatory steady-state during exercise, thereby allowing investigators to assess the influence of a single menthol exposure on body temperature regulation within the thermoregulatory (compensable) zone. Expired gas was collected during rest ($10^{th}$ and $20^{th}$ minutes) and then again after steady state exercise had been achieved ($35^{th}$ minute to $37^{th}$ minute), and again in the middle ($50^{th}$ minute to $52^{nd}$ minute) and at the end of the test ($70^{th}$ minute to $72^{nd}$ minute) using the Douglas bag method. Retrospective analysis of expired gasses allowed investigators to assess whether participants were working at the same relative intensity during each test.

Participants arrived at the laboratory with their own running shoes and shorts, and were provided with a long sleeve breathable shirt. They were weighed naked, and then weighed again whilst wearing their exercise clothing. They self-inserted a rectal thermistor. Eight skin thermistors were secured to the body (chest, scapula, biceps, hand, quadriceps, hamstring, shin and foot). Ventilated sweat capsules were placed on the lower back and

forehead of participants, and a heart rate monitor was placed around their chest. Upon entering the chamber they were instrumented with laser Doppler probes placed on the left index finger, forearm, right thigh, and at the lower back. $\overline{T}_b$ was calculated from $\overline{T}_{msk}$ and $T_{re}$ (Burton, 1935). Perceptual measures of RPE, TC, TS and irritation were recorded every 5 minutes. As a result of the participants wearing clothing during exercise in warm, humid conditions (which may increase skin temperature), and spraying the participants clothing with a cooling solution (which may reduce skin temperature), $\overline{T}_{msk}$ may not be uniform and may require a greater number of sites to reliably calculate it than the commonly used four site formula recommended by Ramanathan (1964) during exercise in the heat. So, to measure $\overline{T}_{msk}$ accurately, more skin sites were required outside of the clothed, sprayed area. Three sites were measured in the area covered by clothing and sprayed, and the remaining five sites were unclothed and unsprayed. $\overline{T}_{msk}$ was calculated using an eight site weighted formula developed by Olesen (1984) (see general methods for formula), who used stepwise regression to identify the eight highest correlated sites used for predicting $\overline{T}_{msk.}$ The eight site formula correlates very highly ($R^2$ = 0.98) with formulae using 14 skin sites in temperatures ranging from 0 to 40 °C, during different activities with different clothing insulations (Olesen, 1984).

***Analyses***

A two-way repeated measure ANOVA, by spray group and time (and interaction) was used to assess parametric data. Non-parametric data were analysed using Friedman's one-way repeated measures ANOVA, and reported in median (range) scores. The alpha level was set at 0.05, unless otherwise specified. Skin temperature measurement was not complete during one test in one participant due to a faulty recording device, so $\overline{T}_{msk}$ and $\overline{T}_b$ have a sample size of 11. Perceptual scores were not collected from one participant at the beginning of one test. As the statistical package cannot accommodate missing data, those scores were omitted; hence, TS, TC, IRR and RPE feature a sample size of 11. Lastly, sweat onset was not observed in one participant given the criteria (*i.e.* an increase of sweat rate two SD above the mean), so those data, in addition the missing $\overline{T}_{msk}$ data, left a sample size of 10 for assessing sudomotor function.

## Results

### *Environmental conditions*

Environmental temperature and rh did not significantly differ by spray group ($P > 0.05$). Overall mean (SD) dry air, globe and wet bulb temperatures were 30.2 (1.1) °C; 30.1 (1.0) °C; and 26.3 (0.9) °C respectively. Mean (SD) relative humidity was 72.2 (3.4) %.

### *Measures of work-rate*

Table 2 shows oxygen consumption measures during exercise, by spray group. A one-way ANOVA showed no difference by spray group ($P > 0.05$) in RER, $\dot{V}_{E,}$ $\dot{V}O_2$, or $\dot{V}CO_2$.

**Table 2**. Mean (SD) oxygen consumption during exercise by spray group ($n = 12$).

| Measure | CON | $M_{0.05\ \%}$ | $M_{0.2\ \%}$ |
|---|---|---|---|
| $\dot{V}_E$ (L·min$^{-1}$) | 49.2 (6.1) | 49.3 (6.3) | 49.1 (4.3) |
| $\dot{V}O_2$ (L·min$^{-1}$) | 2.1 (0.3) | 2.0 (0.3) | 2.1 (0.3) |
| $\dot{V}CO_2$ (L·min$^{-1}$) | 2.0 (0.2) | 1.9 (0.3) | 2.0 (0.3) |
| RER | 0.9 (0.1) | 0.9 (0.1) | 0.9 (0.1) |

Overall group mean (SD) heart rate remained stable at 80 (11.1) beats · min$^{-1}$ during each resting phase and rose to 162 (17.4) beats · min$^{-1}$ by the 45$^{th}$ minute (end) of exercise. A two-way ANOVA showed a difference in HR over time ($P < 0.0001$) and by spray group ($P = 0.028$), with no interaction ($P > 0.05$), but *post-hoc* testing could not detect the direction of effect.

At the onset of exercise, RPE was described as 'light', numerically equivalent to 10 across all groups. After 45 minutes of exercise, RPE approached 'somewhat heavy' to 'heavy' (around 14.5 on the RPE scale). Friedman's One-way ANOVA showed no difference by spray group ($P > 0.05$). The median (range) RPE over 45 minutes of exercise at $PO_{45\ \%}$ for CON, $M_{0.05\ \%}$ and $M_{0.2\ \%}$ was 13 (7 to 19), 13 (6 to 18) and 13 (6 to 20) respectively.

### *Rectal temperature*

Figure 21 shows mean $T_{re}$ during rest and exercise by spray group. A two-way ANOVA showed a difference by time ($P < 0.0001$), and spray group ($P < 0.0001$), and an interaction ($P < 0.034$). *Post-hoc* testing showed that $M_{0.2\ \%}$ had a greater $T_{re}$ from the 40$^{th}$ minute onwards compared to CON, and from the 50$^{th}$ to the 60$^{th}$ minute compared to $M_{0.05\ \%}$ ($P < 0.05$). A one-way ANOVA of the $\Delta T_{re}$ from minute 15 to 75 showed a difference by spray group ($P = 0.029$). *Post-hoc* testing showed that $M_{0.2\ \%}$ induced a greater $\Delta T_{re}$ (1.1 [0.3] °C) than CON (0.9 [0.3] °C) ($P < 0.05$), but not compared to $M_{0.05\ \%}$ (0.9 [0.3] °C) ($P > 0.05$).

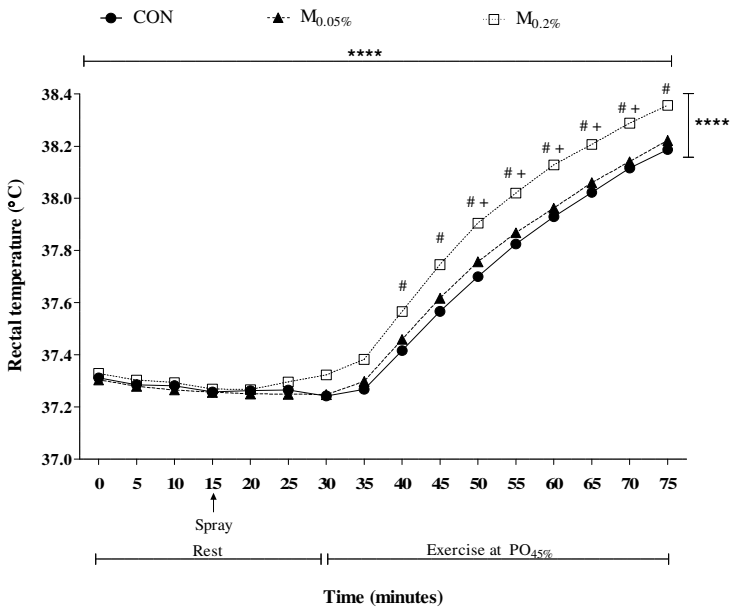


**Figure 21**. Mean rectal temperature during exercise and rest by spray group ($n$ =12). Significant difference (****$P < 0.0001$) by time (⊢⊣) and spray group (⌶). *Post-hoc* test: Significant difference between CON and $M_{0.2\ \%}$ (#, $P < 0.05$); between $M_{0.05\ \%}$ and $M_{0.2\ \%}$ (+, $P < 0.05$).

***Mean skin temperature***

Figure 22 shows $\overline{T}_{msk}$ during exercise and rest, by condition. $\overline{T}_{msk}$ differed significantly by time ($P < 0.0001$), but not by group ($P > 0.05$); nor was there an interaction ($P > 0.05$).

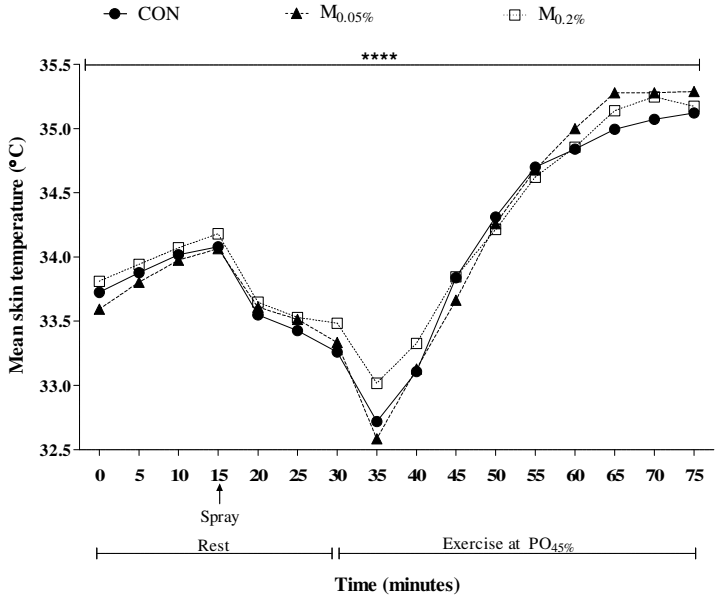


**Figure 22.** Mean skin temperature during exercise and rest, by spray group ($n$ = 11). Significant difference (****$P < 0.0001$) by time (⊢⊣).

***Mean body temperature***

Figure 23 shows $\overline{T}_b$ during exercise and rest, by group. $\overline{T}_b$ differed across time ($P < 0.0001$) and spray group ($P < 0.0001$), with no interaction ($P > 0.05$). The direction of effect could not be detected ($P > 0.05$).

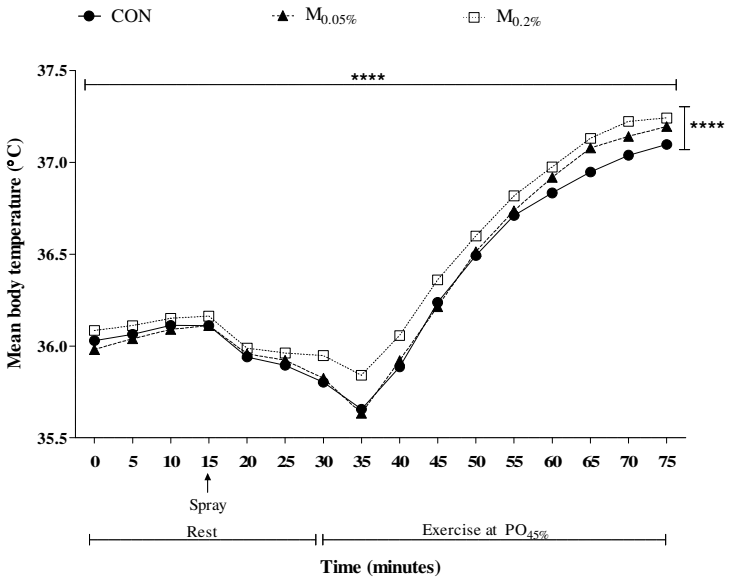


**Figure 23.** Mean body temperature during exercise and rest, by spray group ($n$ = 11). Significant difference (****$P < 0.0001$) by time (⊢⊣) and by spay group (⌶).

***Skin blood flow***

Figure 24 shows the mean change in skin blood flow for the finger (a), forearm (b), lower back (c) and thigh (median shown) (d) during exercise and rest, by spray group. A two-way ANOVA showed that finger SkBF differed by time ($P < 0.0001$) and spray group ($P = 0.0001$), with no interaction ($P > 0.05$). *Post-hoc* testing showed that 0.2 % menthol spraying lowered SkBF at the 25$^{th}$ minute. Forearm SkBF also differed by time ($P < 0.0001$) and spray group ($P = 0.002$), with no interaction ($P > 0.05$), but the direction of effect was not detected. Back SkBF also differed by time ($P < 0.0001$), but not by spray group ($P > 0.05$), with no interaction ($P > 0.05$). Friedman's ANOVA showed that thigh SkBF differed by spray group ($P = 0.046$), but the direction of effect was not detected.

A one-way ANOVA showed no differences ($P > 0.05$) by spray group in the (mean [SD]) onset time (minutes) of vasodilation at the finger (32.7 [0.2] minutes), forearm (33.6 [0.4] minutes), back (33.0 [0.7] minutes) or thigh (33.9 [0.3] minutes). Nor was there a difference in the $\overline{T}_{msk}$ at the onset of vasodilation ($P > 0.05$) at the finger (33.0 [0.05] °C), forearm (32.9 [0.0] °C), back (33.0 [0.0] °C) or thigh (32.9 [0.1] °C).

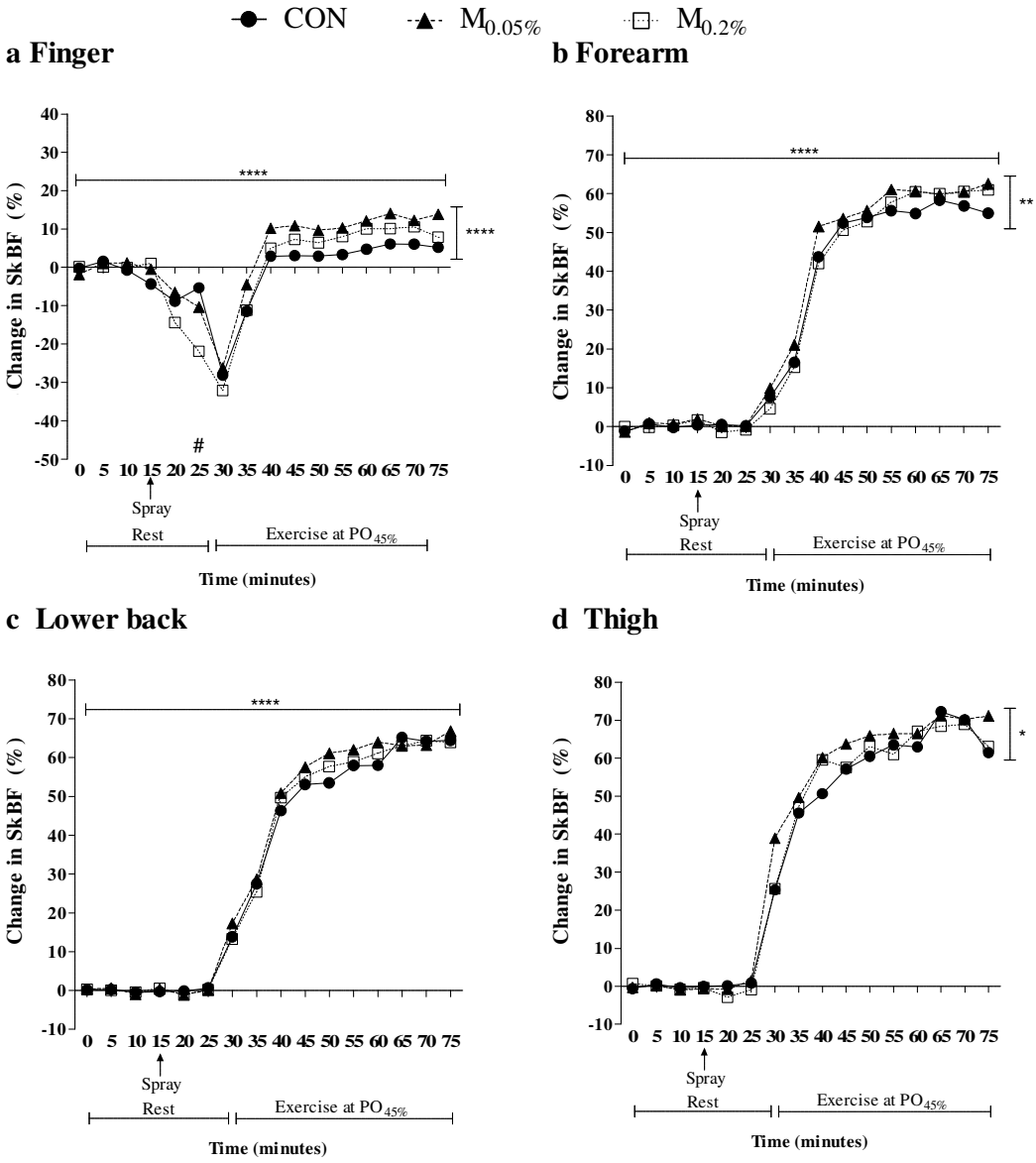


**Figure 24.** Mean skin blood flow for the finger (a), forearm (b), lower back (c) and thigh (median shown) (d) during exercise and rest, by spray group ($n$ = 12). Significant difference (* $P < 0.05$; ** $P < 0.01$, ****$P < 0.0001$) by time (⊢⊣) and by spray group (⌶). *Post-hoc* test: Significant difference between CON and $M_{0.2\,\%}$ (#, $P < 0.01$).

***Sweat rate***

Figure 25 shows mean sweat rate (SR) at the forehead (a) and lower back (b) during exercise, by spray group. SR differed by time at the forehead and lower back ($P < 0.0001$), but not by spray group, with no interaction ($P > 0.05$).

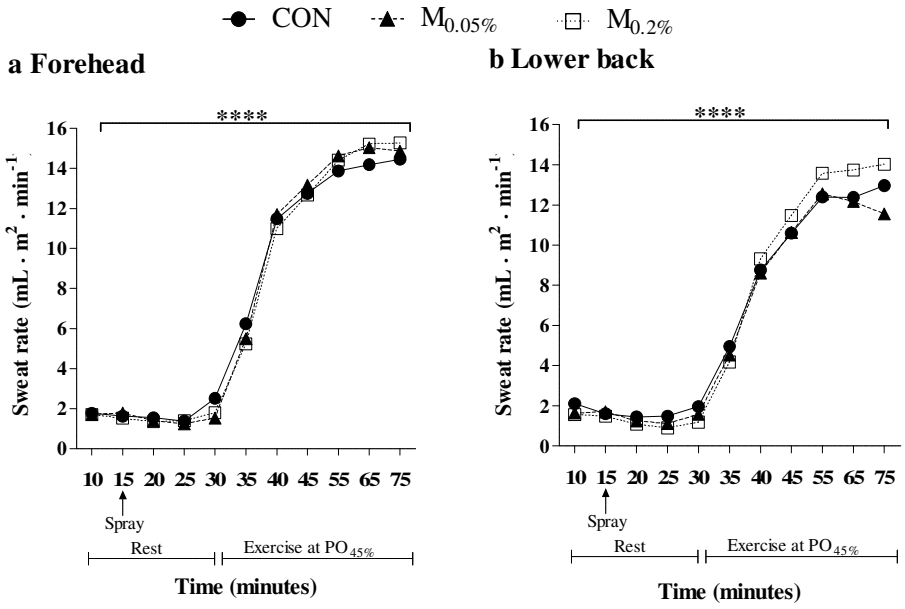


**Figure 25.** Mean sweat rate for the forehead (a) and lower back (b) during exercise and rest, under conditions of water spraying, 0.05 % and 0.2 % menthol spraying ($n$ = 11). Significant difference (****$P$ < 0.0001) by time (⊢⊣).

Table 3 (p.84) shows that the time of onset of lower back sweating (minutes) did not differ by spray group ($P$ > 0.05). However, $T_{re}$ ($P$ = 0.021), $\overline{T}_b$ ($P$ = 0.004) and $\overline{T}_{msk}$ ($P$ = 0.029) recorded at the onset of lower back sweating all differed by spray group. *Post-hoc* testing showed that $T_{re}$ was 0.15 °C higher in $M_{0.2\ \%}$ compared to CON ($P$ < 0.05); similarly, $\overline{T}_b$ was 0.21 °C higher in $M_{0.2\ \%}$ compared to CON ($P$ < 0.05) and also 0.28 °C higher compared to $M_{0.05\ \%}$ ($P$ < 0.05). $\overline{T}_{msk}$ was 0.6 °C higher in $M_{0.2\ \%}$ compared to CON ($P$ < 0.05).

Table 3 also shows that the time of onset of forehead sweating (minutes) did not differ by spray group ($P$ > 0.05). But, $T_{re}$ ($P$ = 0.011), $\overline{T}_b$ ($P$ = 0.003) and $\overline{T}_{msk}$ ($P$ = 0.023) recorded at the onset of forehead sweating all different by spray group. *Post-hoc* testing showed that $T_{re}$ was 0.15 °C higher in $M_{0.2\ \%}$ compared to CON ($P$ < 0.05) and 0.12 °C higher compared $M_{0.05\ \%}$ ($P$ < 0.05); similarly, $\overline{T}_b$ was 0.2 °C higher in $M_{0.2\ \%}$ compared to CON ($P$ < 0.05) and also 0.3 °C higher compared to $M_{0.05\ \%}$ ($P$ < 0.05); lastly, $\overline{T}_{msk}$ was 0.6 °C higher in $M_{0.2\ \%}$ compared $M_{0.05\ \%}$ ($P$ < 0.05) at the onset of sweating. Lastly, there was no difference in the $\Delta T_{re}$ and $\Delta\overline{T}_b$ (calculated from the start of exercise) observed at the onset of sweating at the forehead or lower back, by spray group ($P$ > 0.05) (Table 3).

**Table 3**. Mean (SD) time of onset of sweating (minutes) at the forehead and lower back, and their associated $T_{re}$, $\overline{T}_{msk}$, $\overline{T}_b$, $\Delta T_{re}$ and $\Delta\overline{T}_b$ (°C) under conditions of water spraying, 0.05 % and 0.2 % menthol spraying ($n$ = 10). Significant difference between CON and $M_{0.2\ \%}$ (#, $P < 0.05$) and between $M_{0.05\ \%}$ and $M_{0.2\%}$ (+, $P < 0.05$). Values are calculated from the onset of exercise, at the 30th minute, along the experimental timeline.

| Site | Measure | Water Spray | | 0.05% menthol spray | | 0.2% menthol spray | |
|---|---|---|---|---|---|---|---|
| Lower back | SR onset (min) | 33.5 | (2.8) | 33.7 | (2.5) | 34.4 | (2.9) |
| | $T_{re}$ (°C) | 37.3 | (0.2) | 37.3 | (0.2) | 37.4 | (0.2)$^{\#}$ |
| | $\overline{T}_b$ (°C) | 35.8 | (0.2) | 35.7 | (0.2) | 36.0 | (0.2)$^{\#+}$ |
| | $\overline{T}_{msk}$ (°C) | 33.0 | (0.6) | 32.7 | (0.6) | 33.3 | (0.5)$^{+}$ |
| | $\Delta T_{re}$ (°C) | 0.0 | (0.0) | 0.0 | (0.1) | 0.1 | (0.1) |
| | $\Delta\overline{T}_b$ (°C) | -0.1 | (0.1) | -0.2 | (0.1) | -0.1 | (0.1) |
| Forehead | SR onset (min) | 34.5 | (2.4) | 35.0 | (2.1) | 34.8 | (3.0) |
| | $T_{re}$ (°C) | 37.3 | (0.2) | 37.3 | (0.2) | 37.4 | (0.2)$^{\#+}$ |
| | $\overline{T}_b$ (°C) | 35.7 | (0.2) | 35.7 | (0.2) | 36.0 | (0.2)$^{\#+}$ |
| | $\overline{T}_{msk}$ (°C) | 32.9 | (0.5) | 32.6 | (0.6) | 33.2 | (0.4)$^{+}$ |
| | $\Delta T_{re}$ (°C) | 0.0 | (0.0) | 0.0 | (0.1) | 0.1 | (0.1) |
| | $\Delta\overline{T}_b$ (°C) | -0.1 | (0.1) | -0.1 | (0.1) | -0.0 | (0.1) |

***Upper body thermal comfort***

Figure 26 shows mean upper body thermal comfort during rest and exercise, by group. A two-way ANOVA showed a difference by time ($P = 0.018$) and spray group ($P < 0.0001$), with no interaction ($P > 0.05$). *Post-hoc* testing showed that $M_{0.2\ \%}$ had less comfort during rest after spraying (minute 30, by 2.6 TC units) compared to $M_{0.05\ \%}$ ($P < 0.05$), and greater comfort during exercise (minute 45, by 2.6 TC units), compared to CON ($P < 0.05$).

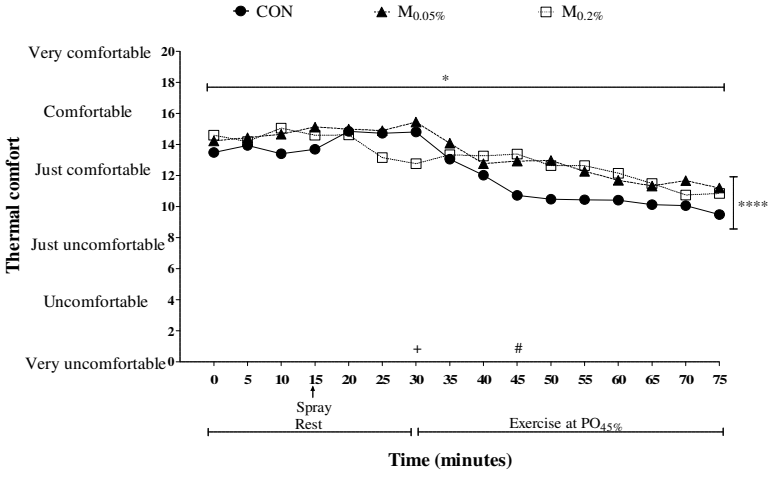


**Figure 26.** Mean thermal comfort for the upper body, by spray condition ($n$ = 11). Significant difference (*$P < 0.05$; ****$P < 0.0001$) by time (⊢⊣) and by spray group (⌶). *Post-hoc* test: Significant difference between CON and $M_{0.2\ \%}$ (#, $P < 0.05$) and between $M_{0.05\ \%}$ and $M_{0.2\ \%}$ (+, $P < 0.05$).

***Upper body thermal sensation***

Figure 27 shows mean upper body thermal sensation during rest and exercise, by group. A two-way ANOVA showed a difference by time ($P < 0.0001$) and spray group ($P < 0.0001$), with an interaction ($P < 0.0001$). *Post-hoc* testing showed that 0.2 % menthol spraying induced significantly cooler sensations within five minutes of spraying compared to CON ($P < 0.001$). 0.05 % menthol spraying induced significantly cooler sensations within 10 minutes of spraying compared to CON ($P < 0.01$). With exercise, participants felt warmer across all groups, but cool sensations lasted the longest, until the 45$^{th}$ minute, following 0.2 % menthol spraying, after which time the conditions were no longer significantly different ($P < 0.05$).

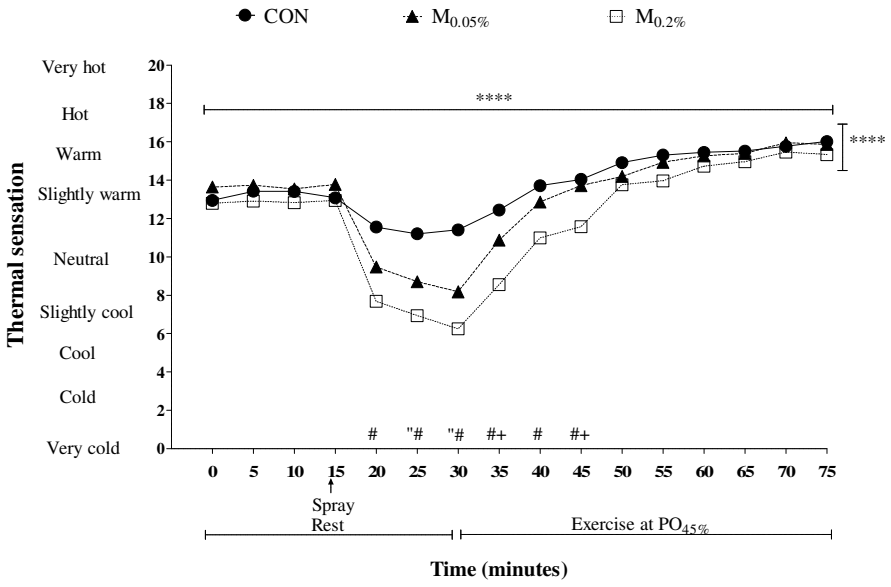


**Figure 27.** Mean thermal sensation for the upper body, by spray group ($n$ = 11). Significant difference (****$P < 0.0001$) by time (⊢⊣) and by spray group (⌶). *Post-hoc* test: Significant difference between CON and $M_{0.2\ \%}$ (#, $P < 0.01$) and between $M_{0.05\ \%}$ and $M_{0.2\ \%}$ (+, $P < 0.05$), and between CON and $M_{0.05\ \%}$ (**''**, $P < 0.01$).

***Irritation***

Figure 28 shows the median irritation score during rest and exercise, by group. Friedman's ANOVA showed a difference by spray condition ($P = 0.0007$). *Post-hoc* testing showed that 0.2 % menthol spraying induced greater irritation than water spraying ($P < 0.001$) and 0.05 % menthol spraying ($P < 0.05$).

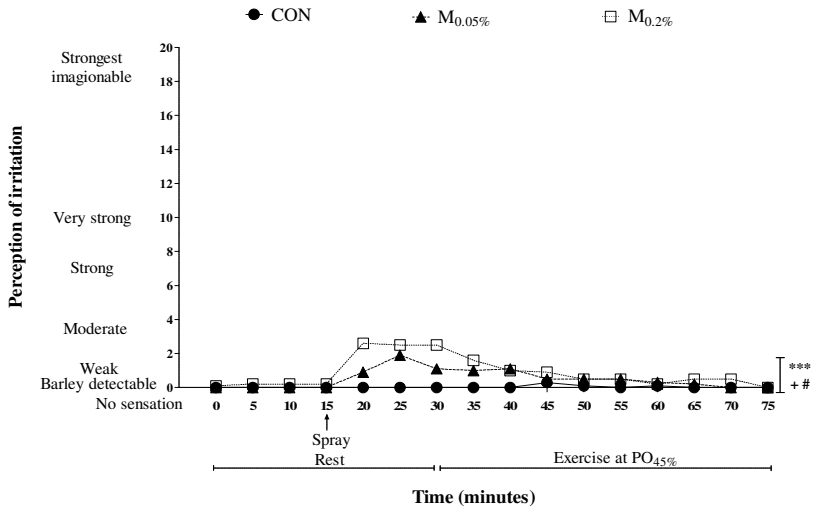


**Figure 28.** Median perceived irritation score by spray group ($n$ = 11). Significant difference (***$P < 0.001$) by spray group (⌶). *Post-hoc* test: Significant difference between CON and $M_{0.2\,\%}$ (#, $P < 0.001$) and between $M_{0.05\,\%}$ and $M_{0.2\,\%}$ (+, $P < 0.05$).

**Discussion and Conclusions**

The first aim of this study was to explore whether lowering the dose of menthol from 0.2 % to 0.05 % could minimise irritation and improve thermal comfort, whilst maintaining cool sensations. The second aim was to characterise the underlying mechanisms driving heat storage following 0.2 % menthol spraying.

The combination of cycle ergometry and heat stress used in this study was sufficient to induce a cardiovascular and thermoregulatory challenge. However, heart rate differed by condition whereby it was higher after 0.2 % menthol spraying, particularly in the early moments of exercise. Although participants were asked to maintain a cycling pace of 60 rpm throughout the entire test (and this was regularly monitored by the investigator), it is possible that the cool sensations imparted by the 0.2 % menthol spray may have enhanced work-rate in the early moments of exercise and in turn raised HR above the other conditions. However, it is also possible that the difference in HR, which did not exceed 5 beats · $min^{-1}$ in the 0.2 % menthol condition, represents normal variation and is perhaps of little practical consequence, particularly as there were no significant differences in any measures of oxygen consumption.

Rectal temperature increased with exercise in all groups, but the elevation was greatest after 0.2 % menthol spraying. Notably, 0.05 % menthol spraying did not induce any

additional heat storage compared to the Control condition. It is worth noting that although elevated, the absolute difference in $T_{re}$ was not greater than 0.2 °C after 0.2 % menthol spraying, and is probably of little practical consequence. Furthermore, $\overline{T}_b$ showed signs of a plateau nearing the end of exercise, indicating that thermal balance was achieved with 0.2 % menthol spraying. Whether this would occur in hotter environments or with more intense exercise requires further study. In any case, 0.2 % menthol spraying influenced body heat storage, and the underlying mechanisms driving this response should be discussed. It has been suggested that cold receptor activation, mediated by menthol, initiates a heat storage response (Kounalakis *et al.,* 2010) by altering both vasomotor and sudomotor function. Skin blood flow was measured at four sites in this study, three of which (excluding the lower back) differed significantly by spray group. At rest, finger SkBF was significantly lower after 0.2 % menthol spraying, but this difference was not observed at the forearm or thigh. SkBF increased at all sites with exercise, but the onset of vasodilation and the associated $\overline{T}_{msk}$ were not influenced by 0.2 % menthol spraying. It seems that vasomotor tone in the finger was most responsive to menthol in this study, and this may point to the underlying mechanism driving heat storage at rest.

When resting in the thermoneutral zone, mean body temperature is regulated by altering skin blood flow, whereby vasoconstriction lowers the amount of thermal energy that is transferred from the body to the environment, and vasodilation increases it (Savage & Brengelmann, 1996). In the conditions of the present study, there was a moderate level of finger skin blood flow when participants were sat resting prior to spraying in 30 °C 70 % rh (Figure 24a). Across all conditions, spraying lowered mean skin temperature and induced vasoconstriction; deep body temperature probably rose inversely with skin temperature (Savage & Brengelmann, 1996), but this cannot be confirmed in the absence of an un-sprayed Control condition. In any case, despite a similar reduction in mean skin temperature across conditions, 0.2 % menthol spraying caused the largest fall in finger skin blood flow, and this coincided with a significant rise in rectal temperature compared to CON and $M_{0.05\ \%}$. Given the only difference between each spray was the dose of menthol it contained, and that the environmental conditions were constant between tests, as were the temperatures of the sprays, the additional vasoconstrictor drive that was observed in the $M_{0.2\ \%}$ condition is probably attributable to the higher 0.2 % dose of menthol. This non-thermal (chemical) factor had the effect of enhancing vasoconstrictor tone at a temperature and level of skin wettedness that would not otherwise induce it, and raises the question of

whether it has the effect of shifting the thermoneutral zone rightward, whereby warmer temperatures (either skin or ambient air) may be required to elicit maximal states of vasodilation? However, this cannot be confirmed from the present study because participants began exercising before the menthol-mediated vasoconstriction had released (Figure 24a). The cascade of events leading to this vasoconstrictor response most probably start with menthol-mediated activation of the cold receptor TRPM8 (McKemy *et al.,* 2002; Peier *et al.,* 2002). Neuronal signals then ascend the spinal cord to excite dis-inhibitory neurons in the hypothalamus that in turn excite pre-motor and pre-ganglionic neurons, which increase vasoconstriction in skin blood vessels (Morrison & Nakamura, 2011). This has the effect of lowering thermal exchange between the skin and the environment, thereby encouraging an accumulation of body heat during rest.

With these initial conditions, $T_{re}$, $\overline{T}_b$ and $\overline{T}_{msk}$ were already visibly elevated before the start of exercise in the 0.2 % menthol group. This explains why those measures were subsequently elevated when sweating had begun shortly after exercise. But importantly, there was no significant difference in the onset time of forehead or lower back sweating, nor was there a difference in the $\Delta T_{re}$ or $\Delta \overline{T}_b$ observed at the onset of sweating between groups. The onset of sweating has been shown to be delayed by skin cooling, but not by reductions in skin blood flow *per-se* (Wingo *et al.,* 2010), so it is possible that the onset of sweating was delayed equally across all spray conditions due to the evaporative cooling produced from water sprayed on the skin; but 0.2 % menthol spraying had no observable influence on the onset of sweating in this study. Furthermore, there was no difference in the absolute sweat rate between spray conditions.

Taken together, these findings suggest that the heat storage response following 0.2 % menthol spraying was induced by vasoconstriction at rest, rather than by a withdrawal of sudomotor function during exercise. This finding is in contrast to Kounalakis *et al.,* (2010), who reported that menthol raised the $T_{re}$ required to initiate sweating *and* delayed its onset by minutes. The disparity is perhaps due to the difference in dose used or area stimulated between studies. For example, the present study sprayed 0.2 % menthol, which equated to 2.1 mg · 100 $cm^{-2}$, over the upper body, while Kounalakis *et al*., (2010) spread 27.5 mg · 100 $cm^{-2}$ of menthol sediment over the whole body. It is possible that the larger surface area exposed (spatial summation) and the larger dose of menthol contributed to a stronger stimulus for heat storage, but this requires clarification.

One final point on thermoeffector function; given that sweating is initiated when $\overline{T}_b$ exceeds the sweating threshold, which is usually 0.2 °C to 0.5 °C above the resting state (Taylor *et al.,* 2008), it is interesting to note that the $\Delta T_{re}$ (calculated from the onset of exercise) required to initiate sweating was very small across all groups in this study (*i.e.* 0.05 °C), and the $\Delta\overline{T}_b$ required to initiate sweating (also calculated from the onset of exercise) was negative (*i.e.* - 0.1 °C) (Table 3). This may be due to the evaporation of water on the skin after spraying, and perhaps supports the use of water spraying prior to exercise as a means of improving heat loss in warm, humid conditions. But it is also possible that non-thermal factors, such as muscular activity (Yanagimoto *et al.,* 2003) or mental stress (Machado-Moreira & Taylor, 2011) caused the onset of sweating.

The primary aim of this study was to explore whether lowering the dose of menthol from 0.2 % to 0.05 % could minimise perceptions of irritation and improve thermal comfort, whilst maintaining cool sensations. When participants were sprayed with either menthol solution their upper body felt cooler than when they were sprayed with the water solution, but 0.2 % menthol spraying made participants feel cooler than 0.05 % menthol spraying, particularly at the start of exercise. Over time however, the cool sensation (*i.e.* thermal sensation) observed after menthol spraying began to approach that of water spraying (*i.e.* the menthol-mediated cool sensations diminished), albeit more quickly when the low dose was used. In total, thermal sensation was enhanced for 35 minutes following menthol spraying, the last 15 minutes of which were during exercise. These findings are in general agreement with the previous studies in this thesis; however, it is worth noting that in the previous studies 0.2 % menthol was sprayed when exercise had begun and repeatedly thereafter, whilst in the current study menthol was sprayed on the participants 15 minutes prior to exercise, when they were at rest. The choice of pre-exercise spraying was taken to investigate the influence of a single menthol application on resting participants, and although this study does not allow for assessment of the optimal spraying time (*i.e.* rest, versus exercise); the results can be contrasted with the previous findings to provide some clues. It seems that menthol may exert its perceptual influence, regardless of whether deep body temperature is elevated or not; if so, it may be more beneficial to spray it during the later stages of exercise, when individuals feel hotter and more uncomfortable thermally; however, further investigation is required to confirm this. In any case, it seems that reducing the menthol dose from 0.2 % to 0.05 % preserves cool sensations to some degree.

When participants were sprayed with 0.2 % menthol, they noted moderate sensations of irritation that peaked from minutes 20 to 30. The perception of irritation then fell with the onset of exercise, and throughout the exercise period such that by the 50$^{th}$ minute, there was no visible difference between conditions. Although 0.05 % menthol spraying appeared to cause weak sensations of irritation, peaking at the 25$^{th}$ minute, the difference between CON and $M_{0.05\ \%}$ was not significant, and was no longer visible by the 45$^{th}$ minute. It is important to note that even water spraying resulted in 'barely detectable' sensations of irritation; this was perhaps due to the rubbing of wet fabric on the skin surface. Lastly, the irritation observed after 0.2 % menthol spraying was significantly greater than that noted after 0.05 % menthol spraying, thereby confirming the assertion first made by Cliff and Green (1994) that reducing the menthol dose, in this case from 0.2 % to 0.05 %, preserves cool sensations and minimises irritation.

In accordance with the findings from Study two, participants felt greater thermal discomfort following 0.2 % menthol spraying whilst they rested, particularly at the 25$^{th}$ minute. Similarly, the strongest feelings of thermal discomfort coincided with the coolest thermal sensations, but also with the strongest sensations of irritation. As a result, it is not possible to confirm whether irritation primarily reduces thermal comfort, or whether thermal comfort is primarily influenced by thermal sensation (*i.e.* in terms of Cabanac's notion of negative allesthesia [1972]). Although thermal comfort was reduced at rest, it improved significantly with exercise such that 0.2 % menthol spraying resulted in the greatest comfort out of all spray groups, particularly after the 45$^{th}$ minute during exercise. Because 0.2 % menthol spraying induced an elevation in $T_{re}$, it remains possible that the elevations in $T_{re}$ accompanying exercise and/or perceived exertion may have diminished sensations of irritation, allowing for an improvement to TC during exercise with menthol spraying. It is difficult to reconcile the 0.2 % menthol-mediated improvement in thermal comfort during exercise with any of the perceptual findings because participants noted both cool sensations and irritation. 0.05 % menthol spraying did not significantly influence thermal comfort during rest or exercise; visually, it compared with water spraying during rest, but then diverged and seemed to track the 0.2 % menthol spraying condition, which was more comfortable compared to water spraying during the latter stages of exercise. These findings suggest that lowering the dose of menthol from 0.2 % to 0.05 % preserves cool sensations, reduces sensations of irritation, but does not significantly alter thermal comfort during rest or exercise.

Given these findings, the null hypothesis that there will be no difference in physiological responses between the two menthol sprays is rejected, in favour of the alternative hypothesis that 0.2 % menthol spraying results in increased heat storage, while 0.05 % menthol spraying does not. The null hypothesis that there will be no difference in perception between the two menthol sprays is not rejected for RPE and thermal sensation, but rejected for irritation and comfort; whereby 0.2 % menthol reduces comfort during rest and improves it during exercise, but 0.05 % menthol causes no change in thermal comfort. Similarly, 0.05 % menthol induced less irritation than 0.2 % menthol. The alternative hypothesis that both menthol solutions will improve thermal sensation and increase irritation during rest and exercise compared to a water spray is supported. The alternative hypothesis that a menthol spray will reduce thermal comfort during rest compared to a water spray is supported for 0.2 % menthol, and rejected for 0.05 % menthol. Lastly, the alternative hypothesis that menthol will increase heat storage compared to a water spray is supported for 0.2 % menthol, but rejected for 0.05 % menthol.

In summary, the heat storage accompanying 0.2 % menthol spraying was probably induced by vasoconstriction at rest, rather than by a withdrawal of sudomotor function during exercise. 0.05 % menthol spraying did not induce any additional heat storage. Furthermore, lowering the menthol dose from 0.2 % to 0.05 % preserves sensations of coolth, reduces irritation, but does not influence thermal comfort negatively. These findings raise the possibility of using a 0.05 % menthol-based water spray as a perceptual cooling strategy for work or exercise in warm, humid conditions; however, in a real world setting, such a strategy could be employed daily, but the influence of repeated menthol exposure is not known. Before this spray can be recommended, further research into its repeated use is needed.

# Chapter 7

# Study 4: The influence of repeated 0.05 % and 0.2 % menthol exposure on thermoregulation and perception during rest and exercise.

## Introduction

Study two showed that adding 0.2 % menthol to a water spray enhances cool sensations, but increases sensations of irritation compared to a Control spray, which may prevent clear improvements in thermal comfort; furthermore, the 0.2 % menthol spray encouraged heat storage. Study three attributed this heat storage to an enhanced vasoconstrictor tone at rest, rather than by a withdrawal of sudomotor function during exercise. Study three also showed that lowering the menthol dose from 0.2 % to 0.05 % preserved sensations of coolth, reduced irritation, but did not influence thermal comfort negatively during rest or exercise; furthermore, there was no observable heat storage following its use. Taken together, these findings raise the possibility of using a 0.05 % menthol-based water spray as a cooling strategy for work or exercise in the heat. But such an intervention would be used entirely at the users' discretion, perhaps daily; and the influence of repeated daily menthol exposure on perception and thermoregulation is unclear. It is important to clarify whether cool sensations habituate after its repeated use.

The influence of repeated menthol exposure on perception has received little attention, and those studies which have been carried out have separated menthol exposures (oral cavity) by minutes, not hours or days (Cliff & Green, 1994; 1996). Given the paucity of research in this area, studies assessing cold adaptation in humans might give clues about the repeated influence of menthol on thermal sensation. A single exposure to menthol is perhaps similar to a single cold exposure in that each gives rise to sensations of coolth. The distinction being that menthol achieves this by direct stimulation of the TRPM8 cold receptor without changing skin temperature (McKemy *et al.,* 2002; Peier *et al.,* 2002), whilst a cold exposure achieves this sensation by first lowering skin temperature, which increases the firing rates of cold receptors and brings about cool sensations. With this distinction in mind, repeated exposures to either cold air (Makinen *et al.,* 2006; Leppaluoto *et al.,* 2001; Bruck *et al.,* 1976) or water (Smolander *et al.,* 2004; Golden & Tipton, 1988) have been shown to cause an habituation of cool sensations and/or thermal discomfort. These findings suggest that repeated exposure to menthol may result in an habituation of

thermal sensation, but no studies have investigated the claim. Further research is required to clarify if there is any habituation in the initial perceptual responses to menthol.

From a thermoregulatory perspective, a single exposure to menthol (*i.e.* 0.2 % or greater) represents a sufficiently potent stimulus to disturb thermoregulation, resulting in heat storage, likely through modulation of the thermoeffectors (Study three; Kounalakis *et al.,* 2010). When a stimulus is strong enough to induce a change in homeostasis, adaptation theory suggests that the physiological outcome (*i.e.* heat storage) resulting from the forcing function (*i.e.* menthol exposure) progressively reduces after repeated exposures (*i.e.* habituates) (Tipton *et al.,* 2008). Generally, this often follows from a shift in the deep body temperature at which vasoconstriction, vasodilation, and sweating begins and ends (Tipton *et al.,* 2008). Therefore, repeated exposure to 0.2 % menthol may attenuate the heat storage response, perhaps through a withdrawal of vasoconstrictor tone, and an increase in skin blood flow. Taken together, it remains possible that repeated menthol exposure may result in a perceptual and/or physiological habituation, clarifying this notion is critical in determining the efficacy of any menthol-based cooling strategy.

## Aims and Hypotheses

The first aim of this investigation was to examine whether thermal sensation habituates after repeated exposure to 0.05 % or 0.2 % menthol, and secondly, to identify whether repeated 0.2 % menthol exposure causes an habituation of the heat storage response.

### *Null hypothesis*

1. There will be no habituation of heat storage or thermal sensation following repeated exposure to 0.2 % or 0.05 % menthol.

## Methods

### *Participants*

Twenty-two participants volunteered for this study; their characteristics are shown in Table 4. There was no significant difference in participant mass or height between conditions ($P > 0.05$), however participants in CON were significantly older than participants in $M_{0.05\%}$ and $M_{0.2\%}$ ($P < 0.05$) (*i.e.* mean difference) by 2.0 years (95 % confidence interval [CI]; 0.2 to 3.9 years) and 1.9 years (95 % CI; .1 to 3.7 years) respectively.

**Table 4.** Mean (SD) participant age, height and weight by spray group

| Condition | Age | | Weight (kg) | | Height (m) | |
|---|---|---|---|---|---|---|
| **Water spray (CON, *n* = *6*)** | 21.6 | (1.3) | 78.8 | (5.5) | 1.80 | (0.05) |
| **0.05 % menthol spray ($M_{0.05\ \%}$, *n* = 8)** | 19.6 | (0.9) | 70.5 | (6.5) | 1.78 | (0.08) |
| **0.2 % menthol spray ($M_{0.2\ \%}$, *n* = 8)** | 19.7 | (1.5) | 76.7 | (15.3) | 1.82 | (0.09) |

***Procedure***

Participants were divided into one of three groups; Control (CON, $n$ = 6), 0.05 % menthol ($M_{0.05\ \%}$, $n$ = 8), and 0.2 % menthol ($M_{0.2\ \%}$, $n$ = 8). Each group underwent the same sequence of testing, comprised of 12 laboratory visits (totalling 12 hours) spread over two weeks. Prior to testing all participants completed three thermal sensitivity familiarisation sessions and a peak power test ($PO_{peak}$). Testing always began on Monday with a pre-intervention exercise test ($Ex_1$) and ended on a Friday, with a post-intervention exercise test ($Ex_2$). On Tuesday, Wednesday and Thursday participants underwent six resting exposures ($R_{1\text{-}6}$), once in the morning and once in the afternoon of each day, each separated by three hours. The testing schedule is displayed in Table 5.

**Table 5**. Participant testing schedule

| | **Monday** | **Tuesday** | **Wednesday** | **Thursday** | **Friday** |
|---|---|---|---|---|---|
| **AM** | $Ex_1$ | $R_1$ | $R_3$ | $R_5$ | $Ex_2$ |
| **PM** | | $R_2$ | $R_4$ | $R_6$ | |

***Exercise sessions ($Ex_1$ and $Ex_2$)***

Exercise testing was undertaken on Monday ($Ex_1$) and Friday ($Ex_2$). The environmental temperature in this study (20 °C) was chosen to be 10 °C lower than previous studies (30 °C) to enhance the stimulus for vasoconstriction prior to exercise, and allow observation of the onset of vasodilation during exercise. Each participant entered the environmental chamber (19.5 °C [0.7] °C; 61.5 % [10.4] % rh) wearing a long sleeve breathable shirt, shorts, training shoes and socks, and remained seated at rest on a cycle ergometer for 10 minutes. Participants then underwent either 0.05 % or 0.2 % menthol spraying or water spraying, and then remained seated for an additional five minutes. At the 15$^{th}$ minute each participant began to cycle at $PO_{45\ \%}$ until $T_{re}$ was raised by 0.5 °C. At this point the test was terminated. The experimental timeline is displayed in Figure 29.

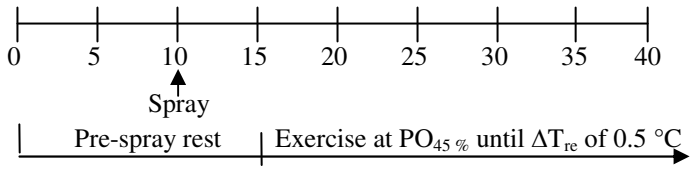


**Figure 29**. Experimental timeline for exercise tests ($Ex_1$ and $Ex_2$)

During each exercise test, participants arrived at the laboratory, were weighed naked (before and after testing) and self-inserted a rectal thermistor. Eight skin thermistors were secured at the left chest, right scapula, left biceps, left dorsal hand, right vastus medialus, left hamstring, right tibalis anterior, right dorsal foot. Mean skin temperature ($\overline{T}_{msk}$) was calculated using an eight site weighted formula developed by Olesen (1984), mean body temperature ($\overline{T}_b$) was calculated using the formula by Burton (1935). Participants were further instrumented with a ventilated sweat capsule on the lower back, and a heart rate monitor. Upon entering the chamber, they were instrumented with a laser Doppler fibre optic probe to measure skin blood flow at the left index finger. Measures of thermal sensation and comfort, perceived exertion and irritation were obtained every 5$^{th}$ minute.

***Resting sessions ($R_1$ through $R_6$)***

To provide a stimulus for an habituation whilst avoiding any training effect from multiple exercise sessions, all groups underwent six resting exposures over three days to either a water spray, 0.05 % or 0.2 % menthol spray. Thermal sensitivity, perceptual, and physiological measures were only taken on the first ($R_1$) and fifth ($R_5$) resting exposures. Measures were taken at $R_5$ rather than $R_6$, as $R_5$ took place in the morning, so any comparison between $R_1$ and $R_5$ should not be influenced by circadian variations in body temperature. Rectal, skin, mean skin, and mean body temperatures, heart rate and skin blood flow (but not sweat rate) were recorded as described in $Ex_1$ and $Ex_2$, along with the perceptual measurements. Thermal sensitivity testing was performed before spraying at the 5$^{th}$ minute and after spraying at the 35$^{th}$ minute of $R_1$, and again at the 35$^{th}$ minute in $R_5$. Each participant entered the environmental chamber in a warm, humid climate comparable to previous studies (29.0 [0.5] °C; 54.9 [3.1] % rh) wearing a long sleeved breathable shirt, shorts, training shoes and socks, and remained seated at rest on a stool for 30 minutes. Participants then underwent either 0.05 % or 0.2 % menthol or water spraying and remained seated for an additional 30 minutes. At this time the test was terminated. The experimental timeline is displayed in Figure 30.

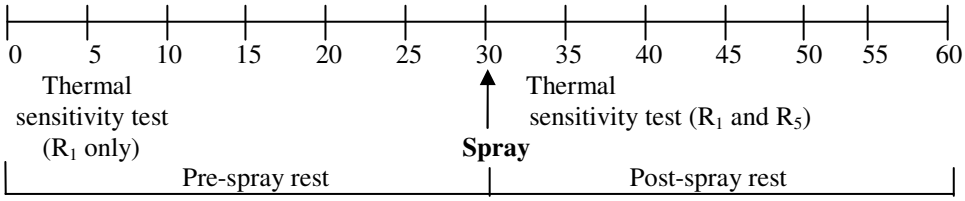


**Figure 30**. Experimental timeline for resting tests ($R_1$ to $R_6$)

Although menthol is best known for its influence on thermal sensation, Green (1992) has shown that it may suppress sensations of forearm warmth. This raises the possibility that individuals who are exposed to menthol in hot environments may not perceive temperatures that, although are not extreme enough to cause tissue damage, may represent a dangerous thermoregulatory challenge. To this end, participants' ability to detect warm temperature stimuli on the forearm was assessed using a thermal sensitivity tester. Three familiarization trials were undertaken prior to the start of testing in order to account for any learning effect (Golja *et al.,* 2003).

***Analyses***

Habituation of a response was judged to occur when its scores diminished over the testing week. Specifically, evidence of habituation would be found if $Ex_1$ significantly differed from $Ex_2$, or if $R_1$ differed from $R_5$. In this way, parametric data were assessed using a two-way repeated measure ANOVA by spray group (CON, $M_{0.05\ \%}$, $M_{0.2\ \%}$) and time ($Ex_1$ vs. $Ex_2$, or $R_1$ vs. $R_5$), with an interaction assessed between the two factors. Non-parametric data were analysed using the Wilcoxon matched-pairs sign rank test within each spray group (*e.g.* CON $Ex_1$ vs. CON $Ex_2$), with a correction for multiple comparisons, and median (range) scores are shown. The alpha level was set at 0.05, unless otherwise specified. Minute-by-minute data were not analysed; instead, either a single mean score, or a change ($\Delta$) in an outcome measure over time (*e.g.* mean thermal sensation, or the change in $T_{re}$ during exercise), were calculated from the raw data and subsequently analysed. Both the minute-by-minute data and the analysed data (either the mean or change) are shown, along with the time points analysed, in each figure to aid clarity. For the exercise sessions, all data were displayed and analysed up to the $40^{th}$ minute, as all participants experienced a change in $T_{re}$ of at least 0.5 °C by this time. Resting data ($R_1$ and $R_5$) were compared over the last 30 minutes of testing.

**Results**

This section is divided in two parts; the first (Part A) will present data from the exercise sessions, which compared perceptual and physiological responses between $Ex_1$ and $Ex_2$. The second section (Part B) will present data from the resting sessions, which compared the perceptual and physiological responses between $R_1$ and $R_5$.

**Part A. Exercise sessions (comparing $Ex_1$ and $Ex_2$)**

***Environmental conditions during the exercise sessions***

There was no difference in mean (SD) dry (19.6 °C [0.6] °C) or globe (19.7 °C [0.6] °C) temperatures between $Ex_1$ and $Ex_2$, or by spray group, and no interaction ($P > 0.05$). Wet bulb temperature differed by spray group ($P = 0.0002$) and between $Ex_1$ and $Ex_2$ ($P = 0.016$), with no interaction ($P > 0.05$). *Post-hoc* testing showed that the environmental temperature in both $Ex_1$ and $Ex_2$ were warmer in CON compared to $M_{0.05\ \%}$ and $M_{0.2\ \%}$, by 2 °C ($P < 0.05$). As such, rh also differed by spray group ($P = 0.001$) and between $Ex_1$ and $Ex_2$ ($P = 0.002$), with no interaction ($P > 0.05$). Again, *post-hoc* testing showed that rh in $Ex_1$ and $Ex_2$ was higher in CON compared to $M_{0.05\ \%}$ and $M_{0.2\ \%}$, by 12 % rh ($P < 0.05$).

***Measures of work-rate during the exercise sessions***

Neither the mean (SD) $\dot{V}O_{2peak}$ (48.2 [6.8] mL · $kg^{-1}$ · $min^{-1}$) nor $PO_{peak}$ (322.1 [48.9] w) differed by spray group ($P > 0.05$). Similarly, mean $\dot{V}O_2$ measured just prior to exercise termination did not differ between $Ex_1$ and $Ex_2$, or spray group, with no interaction ($P > 0.05$). The mean (SD) $\dot{V}O_2$ at exercise termination was 32.1 (3.5) mL · $kg^{-1}$ · $min^{-1}$ across all conditions. Heart rate did not differ between $Ex_1$ and $Ex_2$, or by spray group, with no interaction ($P > 0.05$). During rest, heart rate remained stable around 73 (10.3) beats · $min^{-1}$ across conditions, but rose to 147 (14.8) beats · $min^{-1}$ by the end of exercise. RPE was described as ‘very light’ to ‘light’ at the onset of exercise across conditions and ‘heavy’ after 20 minutes of exercise. The mean RPE during exercise did not differ between $Ex_1$ and $Ex_2$, or by spray group, with no interaction ($P > 0.05$). Mean (SD) RPE during 25 minutes of exercise for CON, $M_{0.05\ \%}$ and $M_{0.2\ \%}$ (averaged between $Ex_1$ and $Ex_2$) was 13.0 (2.5), 12.8 (2.0) and 12.0 (2.7) respectively.

***Rectal temperature during the exercise sessions***

Figure 31a shows the mean $T_{re}$ scores by spray group for $Ex_1$ and $Ex_2$, Figure 31b shows the $\Delta T_{re}$ during exercise, from minute 15 to 40. The $\Delta T_{re}$ during exercise appeared greater in $Ex_1$ compared to $Ex_2$ in all groups, but the difference did not exceed 0.1 °C. Indeed, the $\Delta T_{re}$ (Figure 31b) did not significantly differ between $Ex_1$ and $Ex_2$, nor by spray group, with no interaction ($P > 0.05$).

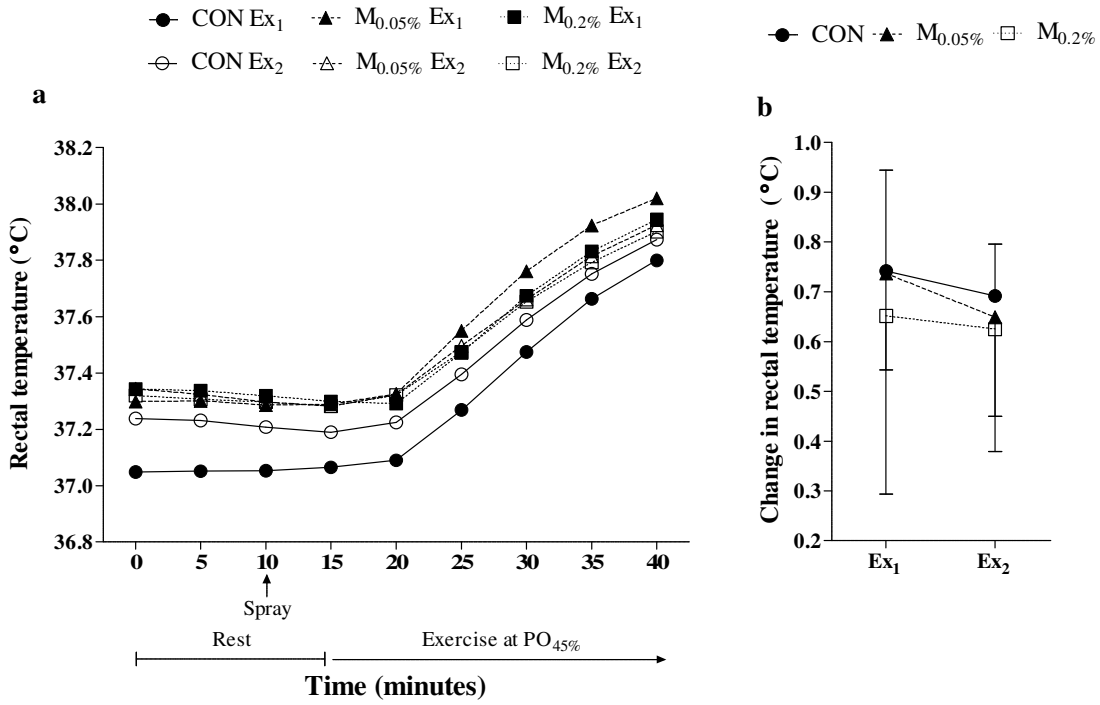


**Figure 31.** Mean rectal temperature during rest and exercise (a) and mean (SD) change in rectal temperature during exercise (b) by spray (CON [$n$ = 6], $M_{0.05\ \%}$ [$n$ = 8], $M_{0.2\ \%}$ [$n$ = 8]) and exercise ($Ex_1$, $Ex_2$) condition.

***Mean skin temperature during the exercise sessions***

Figure 32a shows $\overline{T}_{msk}$ scores by spray group for $Ex_1$ and $Ex_2$, Figure 32b shows the fall in $\overline{T}_{msk}$ post spraying, from time zero to minute 20, and Figure 32c shows the rise in $\overline{T}_{msk}$ with exercise, from minute 20 to 40. $\overline{T}_{msk}$ fell after spraying and rose with exercise equally across all conditions, but did not differ between $Ex_1$ and $Ex_2$, nor by spray group, with no interaction ($P > 0.05$).

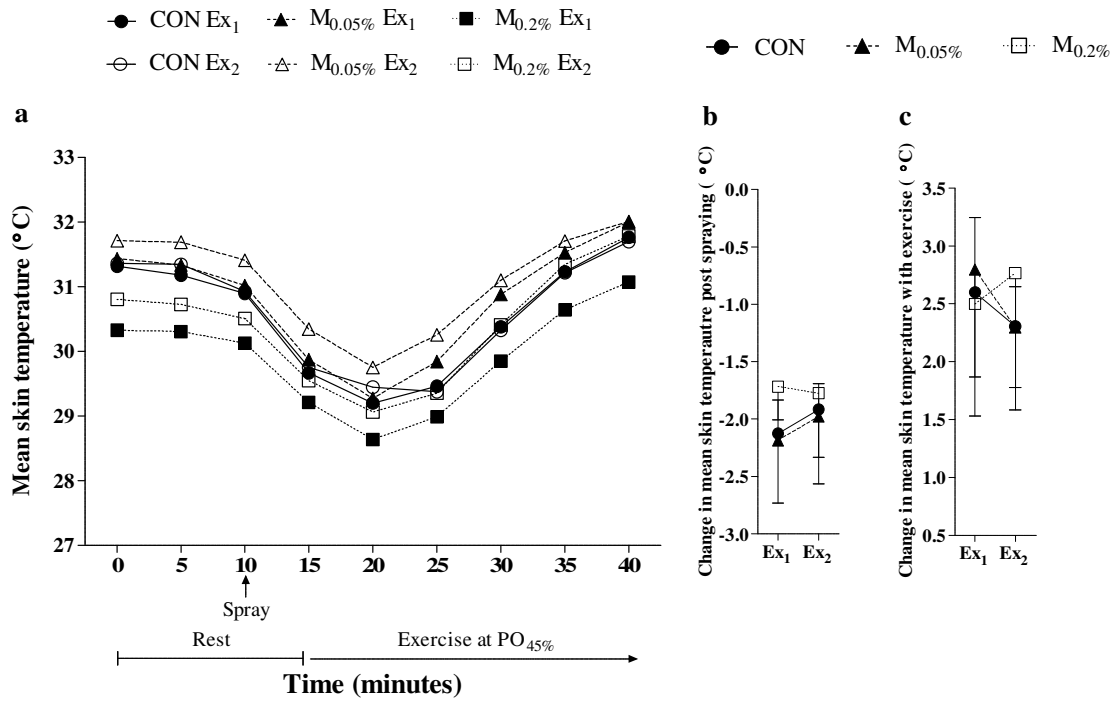


**Figure 32**. Mean skin temperature during rest and exercise (a) and its mean (SD) fall post-spraying (b) and rise with exercise (c) by spray (CON [$n$ = 6], $M_{0.05\ \%}$ [$n$ = 8], $M_{0.2\ \%}$ [$n$ = 8]) and exercise ($Ex_1$, $Ex_2$) condition.

***Mean body temperature during the exercise sessions***

Figure 33a shows $\overline{T}_b$ scores by spray group for $Ex_1$ and $Ex_2$, Figure 33b shows the fall in $\overline{T}_b$ post-spraying, from time zero to minute 20, and Figure 33c shows the rise in $\overline{T}_b$ with exercise, from minute 20 to 40. $\overline{T}_b$ fell after spraying and rose with exercise equally across all conditions, but did not differ between $Ex_1$ and $Ex_2$, nor by spray group, with no interaction ($P > 0.05$).

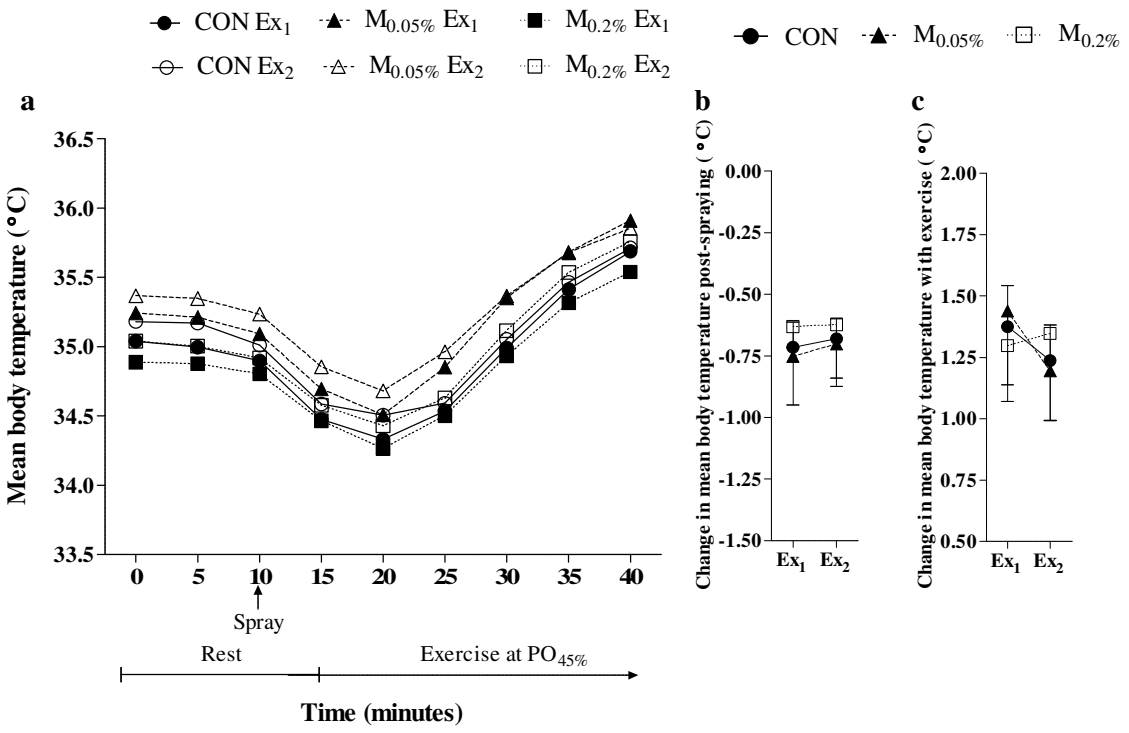


**Figure 33**. Mean body temperature during rest and exercise (a) and its mean (SD) fall post-spraying (b) and rise with exercise (c) by spray (CON [$n$ = 6], $M_{0.05\ \%}$ [$n$ = 8], $M_{0.2\ \%}$ [$n$ = 8]) and exercise ($Ex_1$, $Ex_2$) condition.

***Sweat rate during the exercise sessions***

Figure 34a shows lower back sweat rate by spray group for $Ex_1$ and $Ex_2$, Figure 34b shows $\Delta SR$ during exercise. Sweat rate did not differ between $Ex_1$ and $Ex_2$, nor by spray condition, with no interaction ($P > 0.05$). There were no significant differences in onset of sweating (minutes), or those measures coinciding with the onset of sweating, including; $\overline{T}_{msk}$, $T_{re}$, $\Delta T_{re}$, $\overline{T}_b$, or $\Delta\overline{T}_b$ between $Ex_1$ and $Ex_2$, by spray group, nor was there any interaction ($P > 0.05$), respectively.

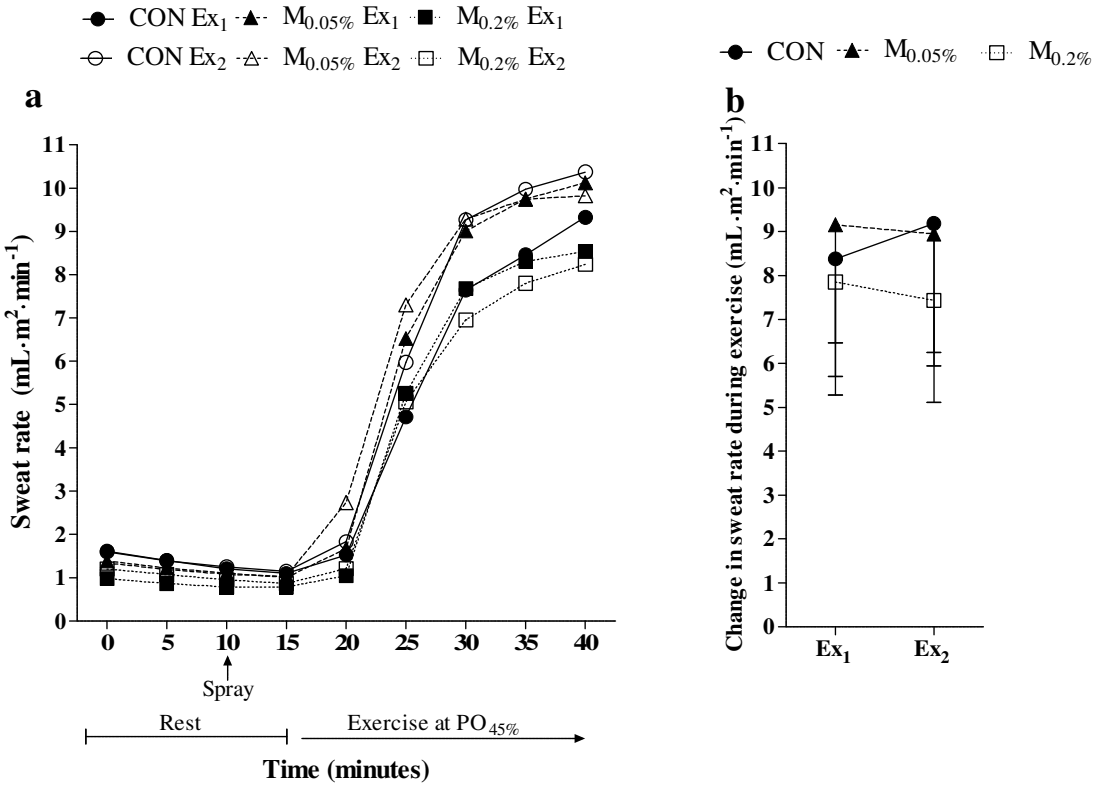


**Figure 34**. Mean lower back sweat rate during rest and exercise (a) and its mean (SD) change during exercise (b) by spray (CON [$n$ = 6], $M_{0.05\ \%}$ [$n$ = 8], $M_{0.2\ \%}$ [$n$ = 8]) and exercise ($Ex_1$, $Ex_2$) condition.

***Skin blood flow during the exercise sessions***

Figure 35a shows finger SkBF by spray group per minute for $Ex_1$ and $Ex_2$, and Figure 35b shows the rise in SkBF from minute 10 to 40. The change in finger SkBF did not differ between $Ex_1$ and $Ex_2$, nor by spray group, with no interaction ($P > 0.05$). There were no significant differences in onset of vasodilation (minutes), or the coinciding hand skin temperature between $Ex_1$ and $Ex_2$, by spray group, nor any interaction ($P > 0.05$).

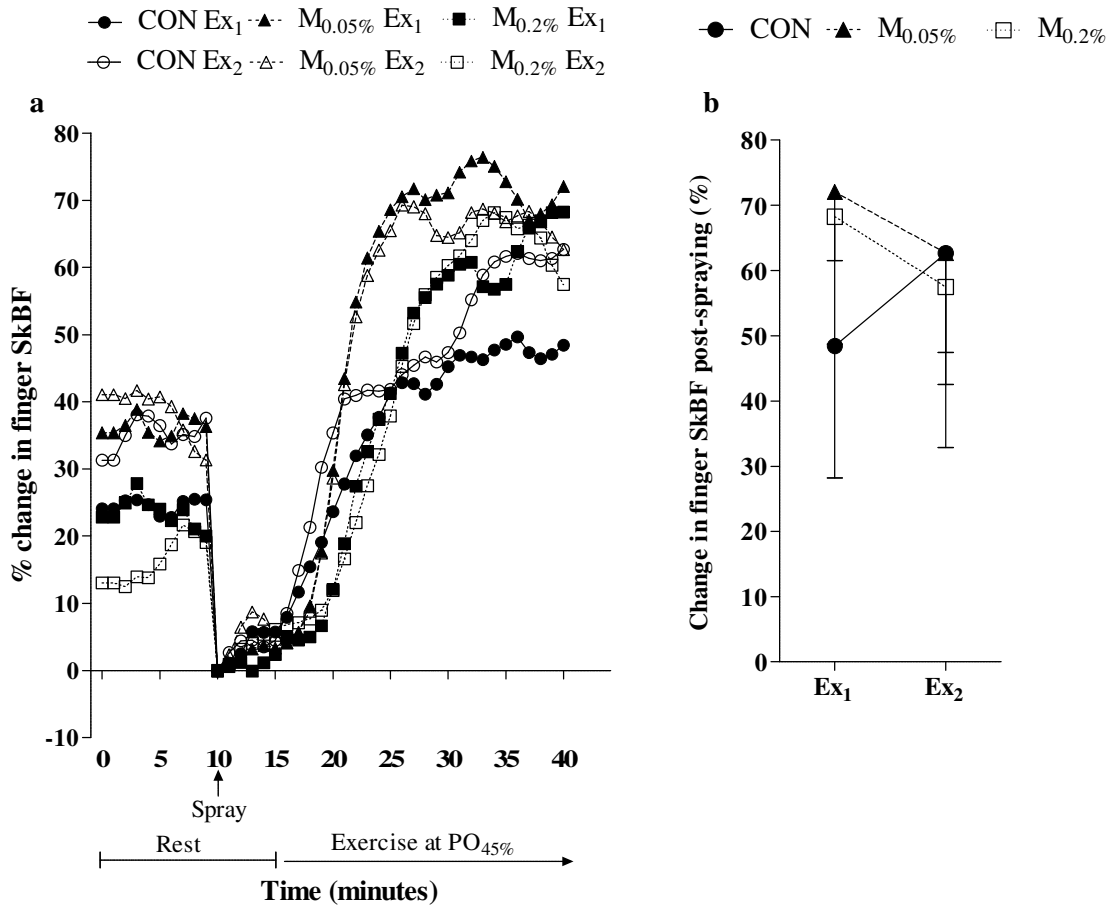


**Figure 35**. Mean finger SkBF by the minute during rest and exercise (a) and its mean (SD) change from the 10th to the 40th minute (b) by spray (CON [$n = 6$], $M_{0.05\,\%}$ [$n = 8$], $M_{0.2\,\%}$ [$n = 8$]) and exercise ($Ex_1$, $Ex_2$) condition.

***Upper body thermal comfort during the exercise sessions***

Figure 36a shows upper body thermal comfort by spray group for $Ex_1$ and $Ex_2$, Figure 36b shows the mean TC score during exercise, from minute 15 to 40. Participants across all conditions felt 'just comfortable' to 'comfortable' prior to spraying. After spraying and with the onset of exercise, TC fell across all conditions such that participants felt 'just uncomfortable' by the end of exercise. Thermal comfort did not differ between $Ex_1$ and $Ex_2$, nor by spray group, with no interaction ($P > 0.05$).

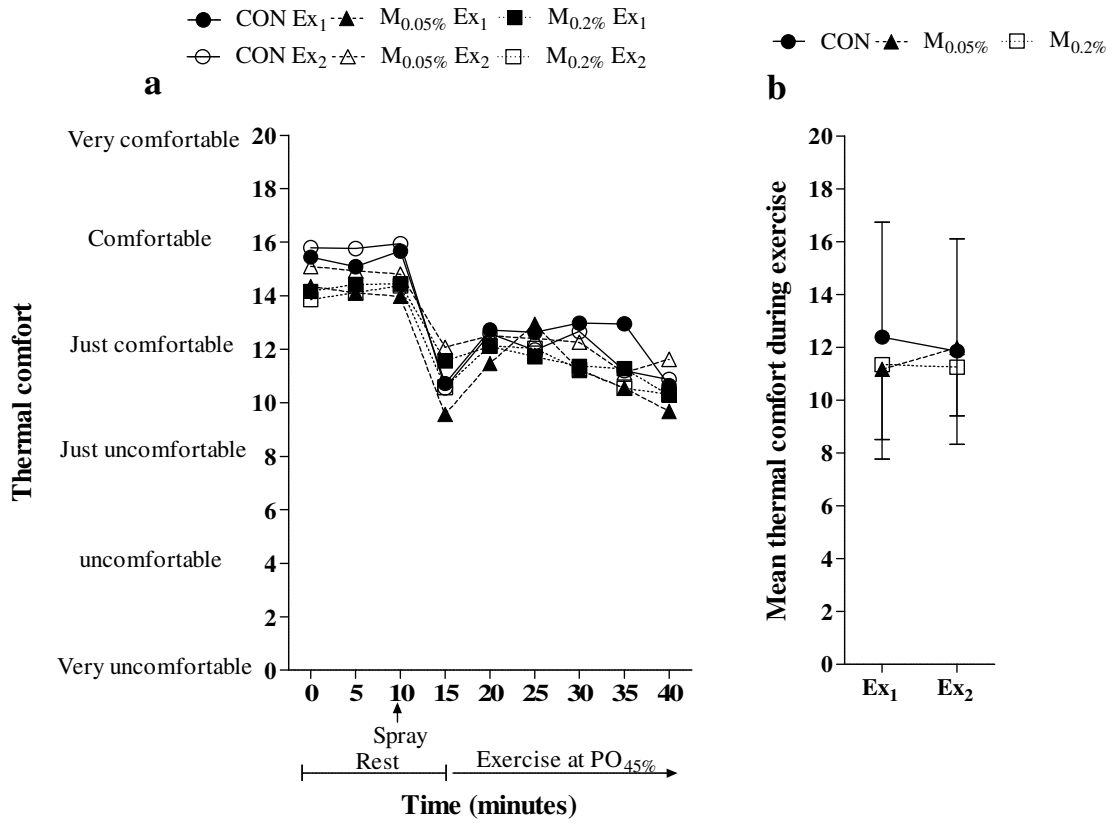


**Figure 36.** Upper body thermal comfort scores during rest and exercise (a) and mean (SD) upper body thermal comfort from the 15th to the 40th minute (b) by spray (CON [$n$ = 6], $M_{0.05\,\%}$ [$n$ = 8], $M_{0.2\,\%}$ [$n$ = 8]) and exercise ($Ex_1$, $Ex_2$) condition.

***Upper body thermal sensation during the exercise sessions***

Figure 37a shows upper body thermal sensation by spray group for $Ex_1$ and $Ex_2$, Figure 37b shows the mean TS score during exercise, from minute 15 to 40. Participants across all conditions felt 'neutral' prior to spraying. After spraying and with the onset of exercise, TS fell across all conditions such that participants felt 'cool' by the 15$^{th}$ minute. All participants felt warmer as exercise continued, but participants in CON appeared to feel warmer than those sprayed with 0.05 % menthol, who in turn felt warmer than those sprayed with 0.2 % menthol. Thermal sensation differed significantly between $Ex_1$ and $Ex_2$ ($P$ = 0.017) and by spray group ($P$ = 0.047), with an interaction ($P$ = 0.015), suggesting that the scores in $Ex_1$ and $Ex_2$ were influenced differently by each spray condition. *Post-hoc* testing showed that 0.2 % menthol spraying induced significantly cooler sensations than Control spraying during $Ex_1$ ($P < 0.01$), but not during $Ex_2$ ($P > 0.05$), indicating an habituation of thermal sensation after repeated exposure to 0.2 % menthol.

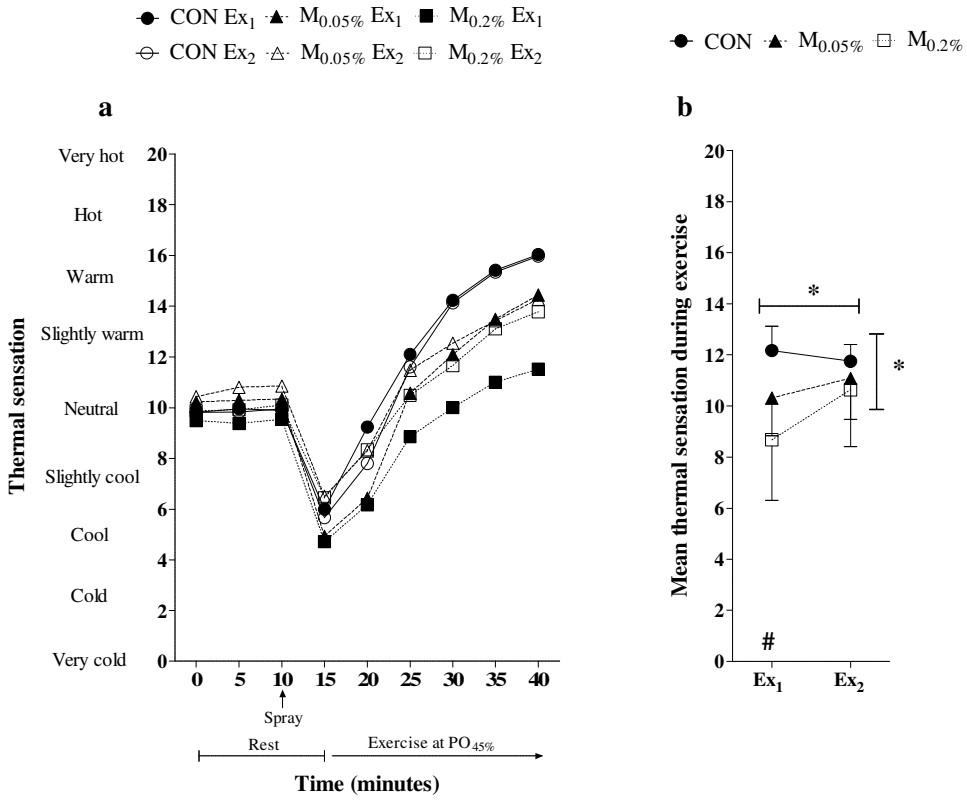


**Figure 37**. Upper body thermal sensation during rest and exercise (a) and mean (SD) upper body thermal sensation from the 15$^{th}$ to the 40$^{th}$ minute (b) by spray (CON [$n$ = 6], $M_{0.05\ \%}$ [$n$ = 8], $M_{0.2\ \%}$ [$n$ = 8]) and exercise ($Ex_1$, $Ex_2$) condition. Significant difference (* $P < 0.05$) between $Ex_1$ and $Ex_2$ (⊢⊣) and by spray condition (⌶). *Post-hoc* test: Significant difference between CON and $M_{0.2\ \%}$ (#, $P < 0.01$).

***Irritation during the exercise sessions***

Figure 38 shows the individual perception of irritation by spray group for $Ex_1$ and $Ex_2$, averaged from minute 15 to 40. The scale has been truncated to show greater detail. A complete version of the scale is shown in Figure 6 (pp. 43) and 28 (pp. 86). Eight participants noted some irritation, four in either menthol spray group. Of these eight, five reported greater irritation during $Ex_1$ compared to $Ex_2$; however, a non-parametric Wilcoxon test showed no difference ($P > 0.05$) in the mean irritation score between $Ex_1$ and $Ex_2$.

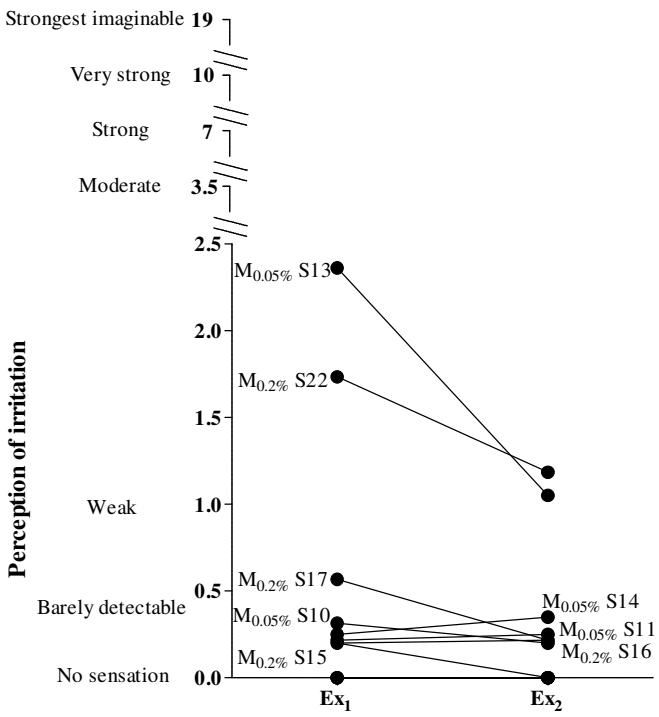


**Figure 38**. Perceived irritation of eight individuals reporting irritation after spraying across exercise conditions ($Ex_1$ and $Ex_2$) ($n = 8$).

**Part B. Resting sessions (comparing $R_1$ and $R_5$)**

***Environmental conditions during the resting sessions***

There was no difference in the mean dry (29.1 [0.5] °C), globe (28.9 [0.5] °C) or wet bulb (22.3 [1.4] °C) temperatures, or rh (54.0 [4.6] %) by spray group, or between $R_1$ and $R_5$, with no interaction ($P > 0.05$).

***Rectal temperature during the resting sessions***

Figure 39a shows the mean $T_{re}$ scores by spray group for $R_1$ and $R_5$, Figure 39b shows the change in $T_{re}$ during the last 30 minutes of rest, from minute 30 to 60. The change in $T_{re}$ did not differ between $R_1$ and $R_5$ ($P > 0.05$), but did significantly differ by spray group ($P = 0.007$), with no interaction ($P > 0.05$). *Post-hoc* testing showed that 0.2 % menthol spraying induced a significant elevation in $T_{re}$ compared to CON and $M_{0.05\ \%}$, during both $R_1$ ($P < 0.05$) and $R_5$ ($P < 0.05$), indicating no habituation of heat storage after repeated exposure to 0.2 % menthol.

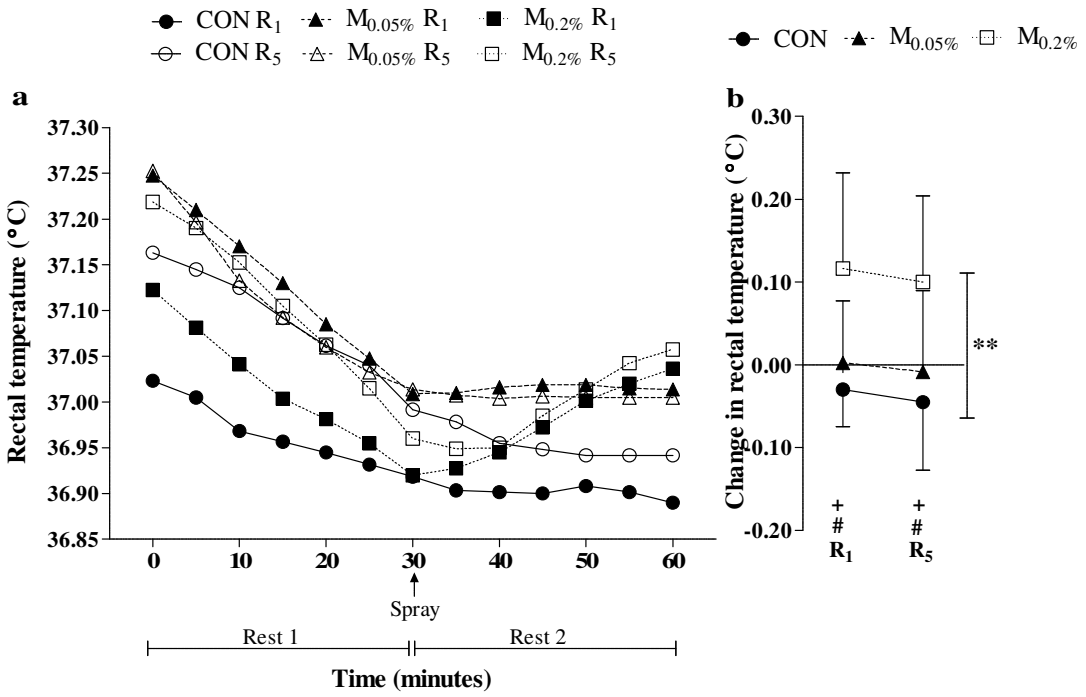


**Figure 39**. Mean rectal temperature during 60 minutes of rest (a) and its mean (SD) change in in the last 30 minutes of rest, post-spraying (b) by spray (CON [$n = 6$], $M_{0.05\ \%}$ [$n = 8$], $M_{0.2\%}$ [$n = 8$]) and resting ($R_1$, $R_5$) condition. Significant difference (** $P < 0.01$) by spray condition (I). *Post-hoc* test: Significant difference between CON and $M_{0.2\ \%}$ (#, $P < 0.05$) and between $M_{0.05\ \%}$ and $M_{0.2\ \%}$ (+, $P < 0.05$).

***Mean skin temperature during the resting sessions***

Figure 40a shows $\overline{T}_{msk}$ scores by spray group for $R_1$ and $R_5$, Figure 40b shows the fall in $\overline{T}_{msk}$ after spraying from minute 30 to 60. The fall in $\overline{T}_{msk}$ did not differ between $R_1$ and $R_5$, nor by spray group, with no interaction ($P > 0.05$).

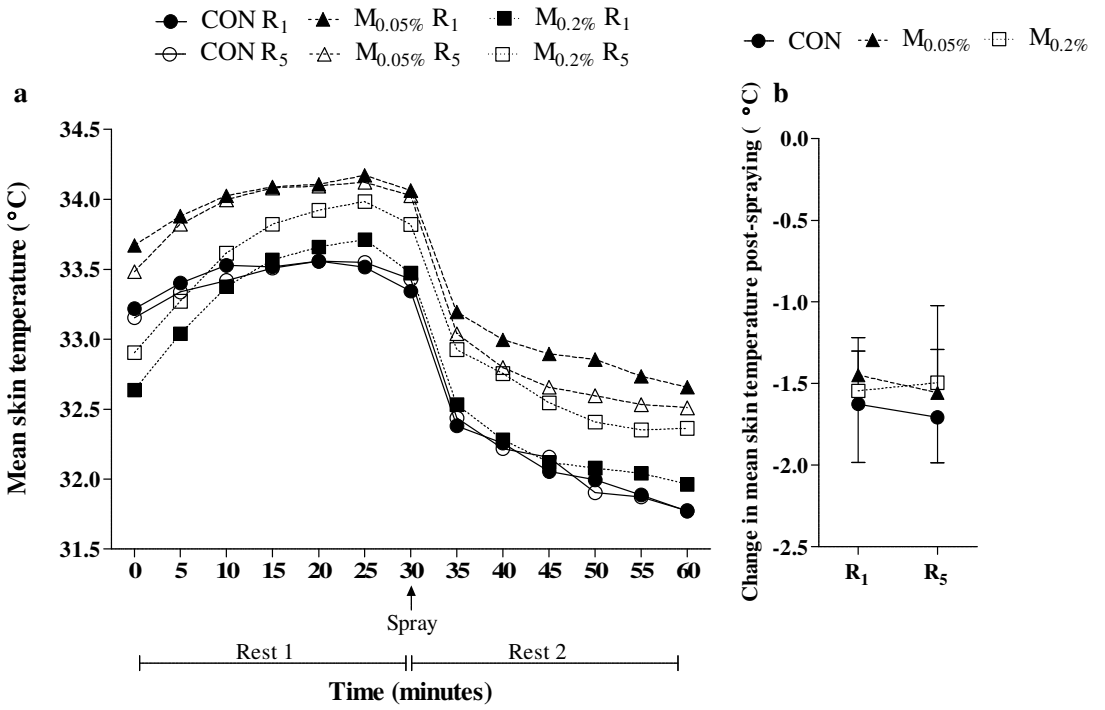


**Figure 40.** Mean skin temperature during 60 minutes of rest (a) and its mean (SD) change in the last 30 minutes of rest, post-spraying (b) by spray (CON [$n = 6$], $M_{0.05\,\%}$ [$n = 8$], $M_{0.2\,\%}$ [$n = 8$]) and resting ($R_1$, $R_5$) condition.

***Mean body temperature during the resting sessions***

Figure 41a shows $\overline{T}_b$ scores by spray group for $R_1$ and $R_5$, Figure 41b shows the fall in $\overline{T}_b$ during the last 30 minutes of rest, from minute 30 to 60. The fall in $\overline{T}_b$ after spraying did not differ between $R_1$ and $R_5$, nor by spray group, with no interaction ($P > 0.05$).

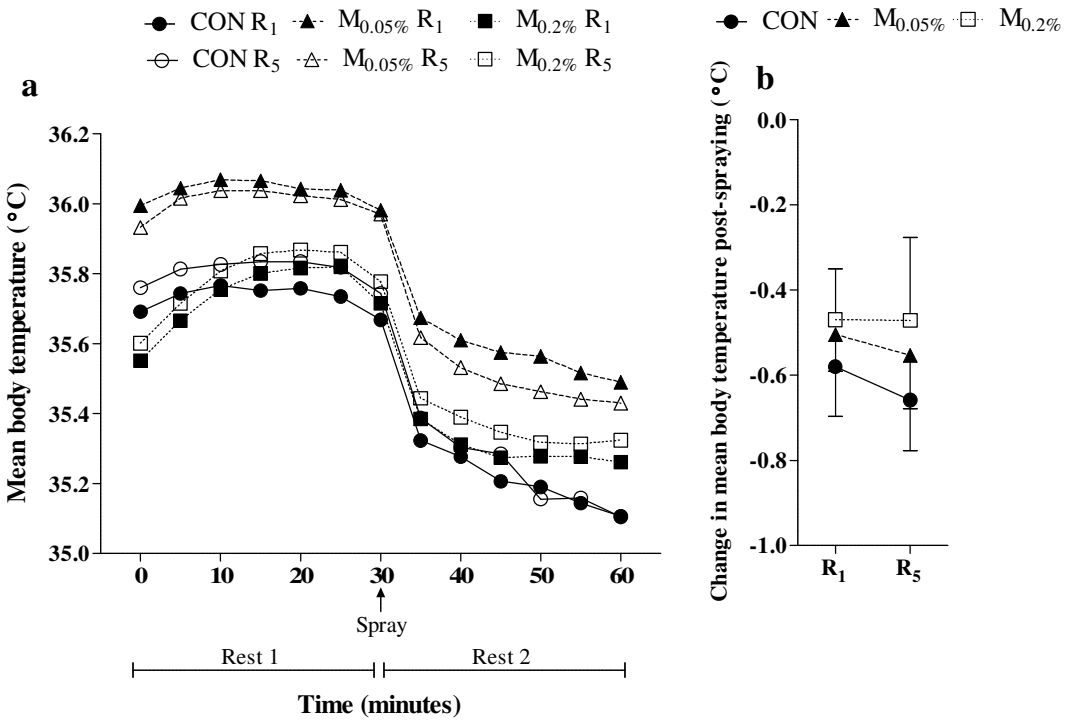


**Figure 41**. Mean body temperature during 60 minutes of rest (a) and its mean (SD) change in the last 30 minutes of rest, post-spraying (b) by spray (CON [$n$ = 6], $M_{0.05\,\%}$ [$n$ = 8], $M_{0.2\,\%}$ [$n$ = 8]) and resting ($R_1$, $R_5$) condition.

***Finger skin blood flow during the resting sessions***

Figure 42a shows finger SkBF by spray group for $R_1$ and $R_5$, Figure 42b shows the mean SkBF, averaged over the last 30 minutes of rest from minute 30 to 60. Mean SkBF (Figure 42b) did not differ between $R_1$ and $R_5$ ($P > 0.05$), but did significantly differ by spray group ($P = 0.002$), with no interaction ($P > 0.05$). *Post-hoc* testing showed that 0.2 % menthol spraying induced a significant reduction in finger SkBF compared to CON during both $R_1$ ($P < 0.01$) and $R_5$ ($P < 0.01$), and compared to $M_{0.05\ \%}$ in $R_5$ ($P < 0.05$). Neither the onset of vasodilation, nor the coinciding skin temperature measured on the back of the hand differed between $R_1$ and $R_5$, or by spray group, with no interaction ($P > 0.05$). These findings indicate no habituation of the enhanced vasoconstrictor response following a single and after repeated 0.2 % menthol spraying.

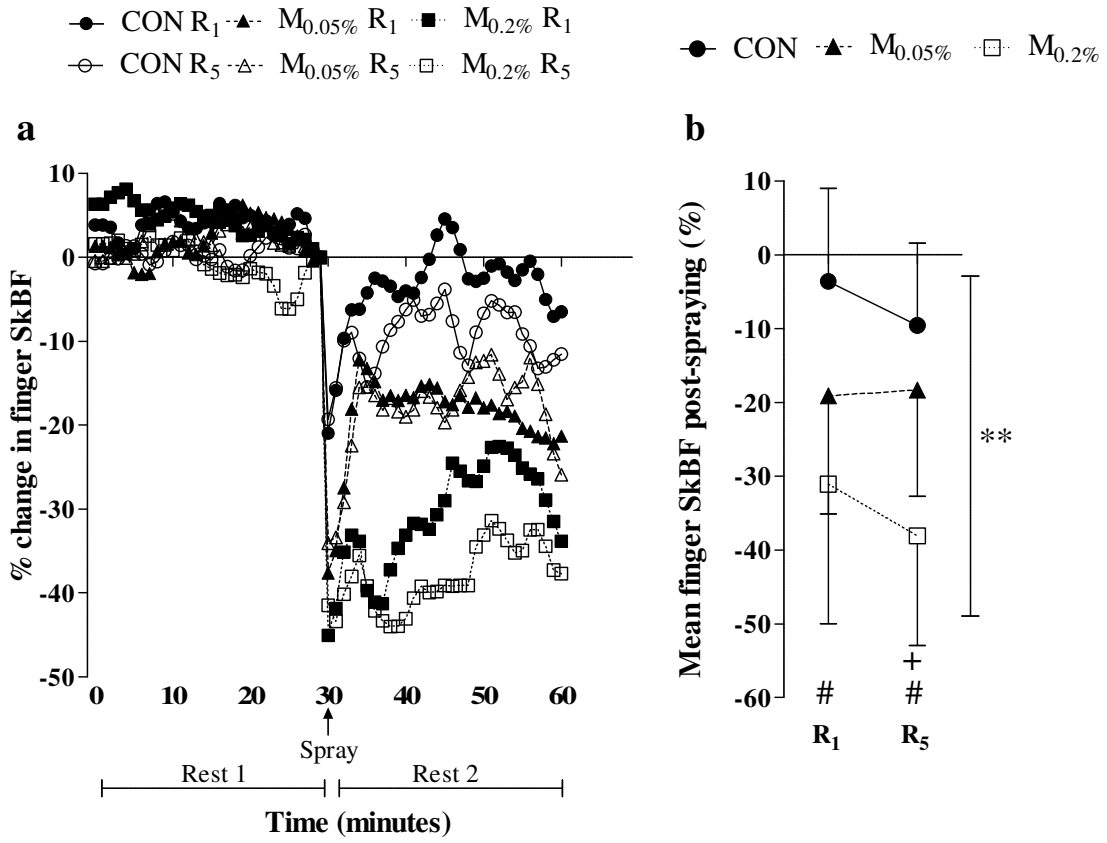


**Figure 42**. Mean finger SkBF during 60 minutes of rest (a) and its mean (SD) score over the last 30 minutes of rest (b) by spray (CON [$n = 6$], $M_{0.05\ \%}$ [$n = 8$], $M_{0.2\ \%}$ [$n = 8$]) and resting ($R_1$, $R_5$) condition. Significant difference (** $P < 0.01$) by spray condition (I). *Post-hoc* test: Significant difference between CON and $M_{0.2\ \%}$ (#, $P < 0.05$) and between $M_{0.05\ \%}$ and $M_{0.2\ \%}$ (+, $P < 0.05$).

***Upper body thermal comfort during the resting sessions***

Figure 43a shows upper body thermal comfort by spray group for $R_1$ and $R_5$, Figure 43b shows the mean TC score from minute 30 to 60. Participants across all conditions felt 'just comfortable' to 'comfortable' prior to spraying. After spraying, TC fell across all conditions, albeit more so with either menthol spray, such that comfort reduced, but did not reach discomfort. Thermal comfort did not differ between $R_1$ and $R_5$, nor by spray group, with no interaction ($P > 0.05$).

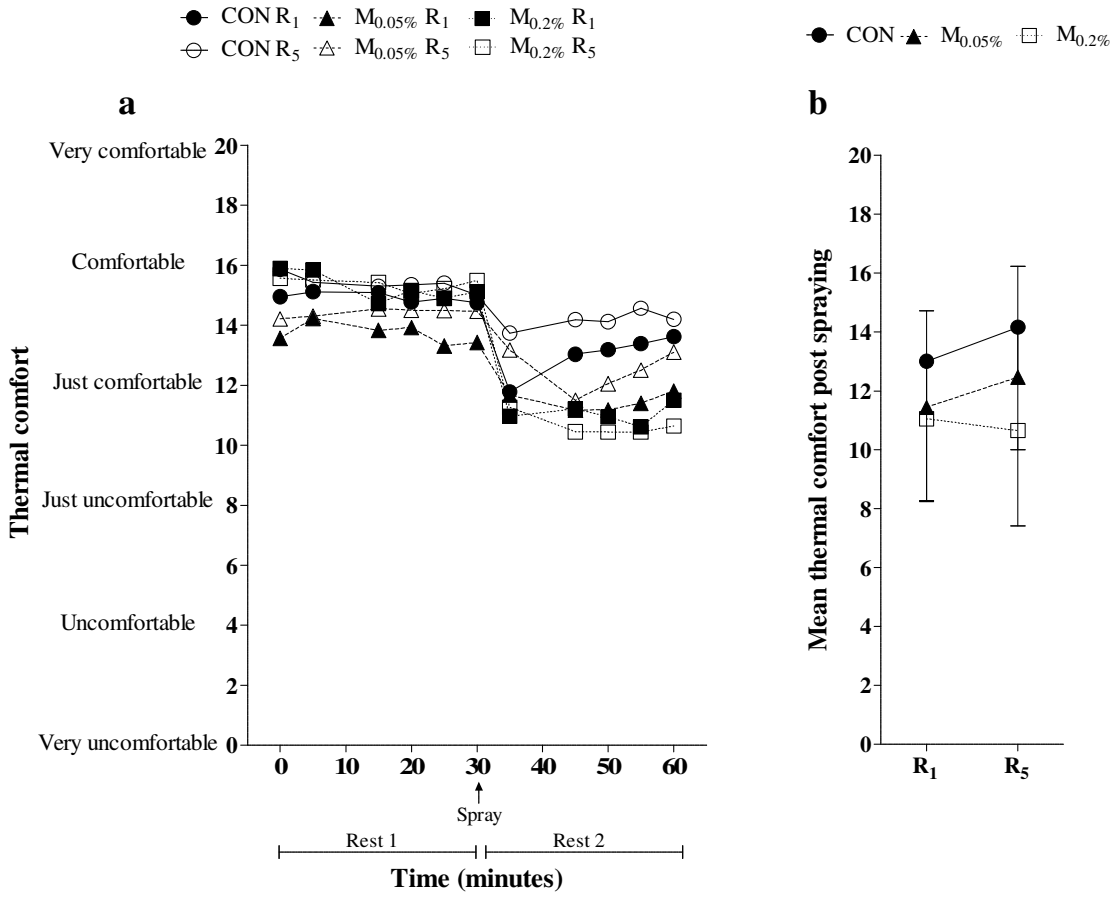


**Figure 43**. Mean upper body thermal comfort during 60 minutes of rest (a) and its mean (SD) score over the last 30 minutes (b) by spray (CON [$n$ = 6], $M_{0.05\ \%}$ [$n$ = 8], $M_{0.2\ \%}$ [$n$ = 8]) and resting ($R_1$, $R_5$) condition.

***Upper body thermal sensation during the resting sessions***

Figure 44a shows upper body thermal sensation by spray group for $R_1$ and $R_5$, Figure 44b shows the mean TS score during the last 30 minutes of rest. Participants across all conditions felt 'slightly warm' to 'warm' prior to spraying. After spraying, TS fell across all conditions such that participants felt 'slightly cool' to 'cool' by the 35$^{th}$ minute. Participants in CON appeared to feel warmer than those sprayed with 0.05 % menthol, who in turn felt warmer than those sprayed with 0.2 % menthol. Thermal sensation differed significantly between $R_1$ and $R_5$ ($P$ = 0.017), but not by spray group ($P$ = 0.08), with no interaction ($P > 0.05$) and the direction of effect could not be determined statistically.

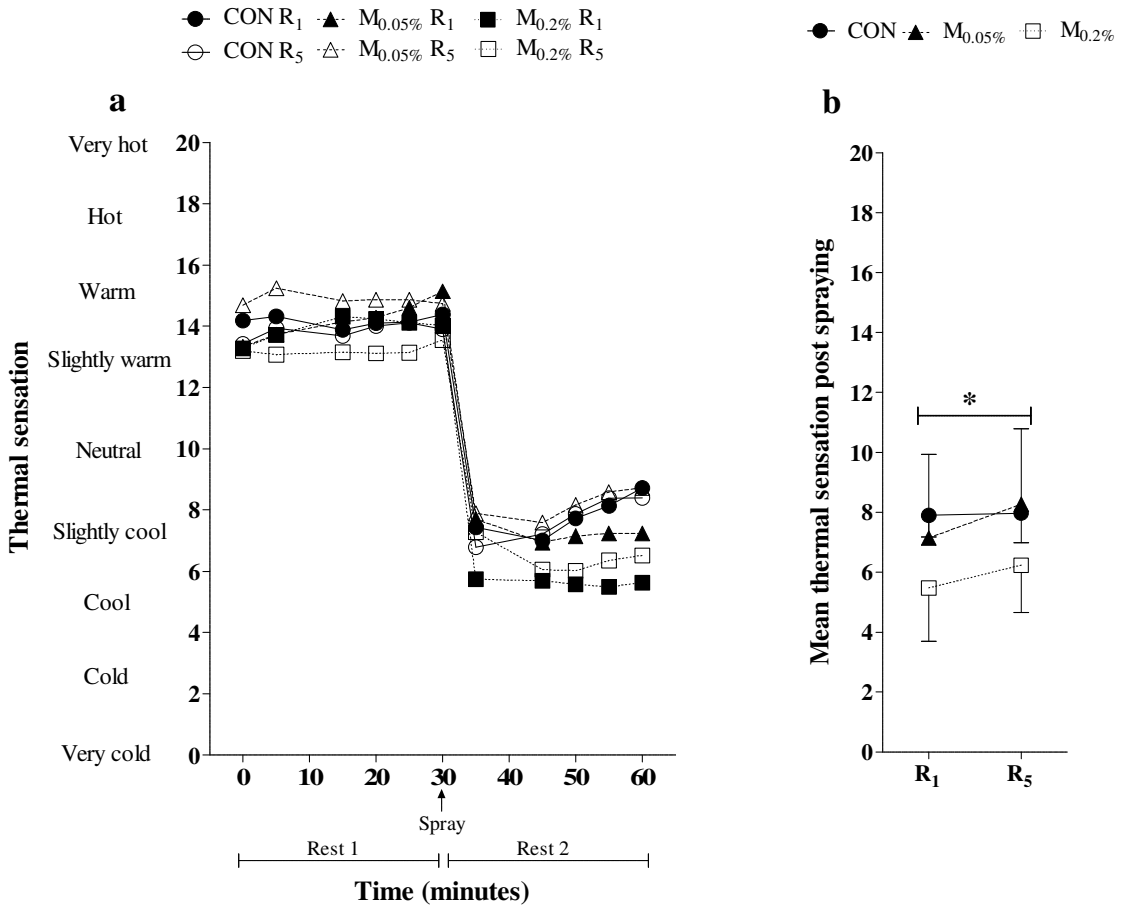


**Figure 44**. Mean upper body thermal sensation during 60 minutes of rest (a) and its mean (SD) score over the last 30 minutes (b) by spray (CON [$n$ = 6], $M_{0.05\ \%}$ [$n$ = 8], $M_{0.2\ \%}$ [$n$ = 8]) and resting ($R_1$, $R_5$) condition. Significant difference (* $P < 0.05$) between $R_1$ and $R_5$ (⊢⊣). *Post-hoc* test: Significant difference between CON and $M_{0.2\ \%}$ (#, $P < 0.05$) and between $M_{0.05\ \%}$ and $M_{0.2\ \%}$ (+, $P < 0.05$).

***Irritation during the resting sessions***

Figure 45 shows individual irritation by spray group for $R_1$ and $R_5$, averaged from minute 30 to 60. Nine participants out of 16 exposed to menthol noted irritation, five in $M_{0.2\ \%}$ and four in $M_{0.05\%}$. Of these nine, six noted greater irritation during $R_1$ than $R_5$; however, a nonparametric Wilcoxon test showed no difference ($P > 0.05$) in the mean irritation score between $R_1$ and $R_5$.

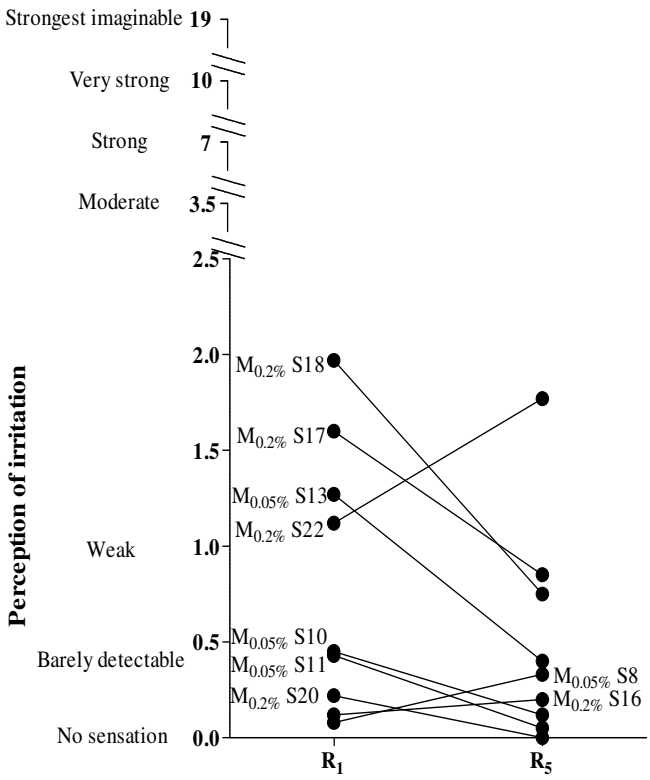


**Figure 45**. Perceived irritation of those individuals noting irritation, by resting conditions ($R_1$ and $R_5$) ($n = 9$).

***Thermal sensitivity during the resting sessions***

Figure 46 shows warm thresholds by spray group measured at baseline, and shortly after spraying in $R_1$, and $R_5$. The warm threshold measured in the first resting test ($R_1$) appeared to increase in $R_1$ post spraying across all groups, but most visibly in $M_{0.2\ \%}$. Over the week, the warm threshold changed little by group. The warm threshold did not differ between $R_1$ and $R_5$ post spraying, nor by spray group, with no interaction ($P > 0.05$).

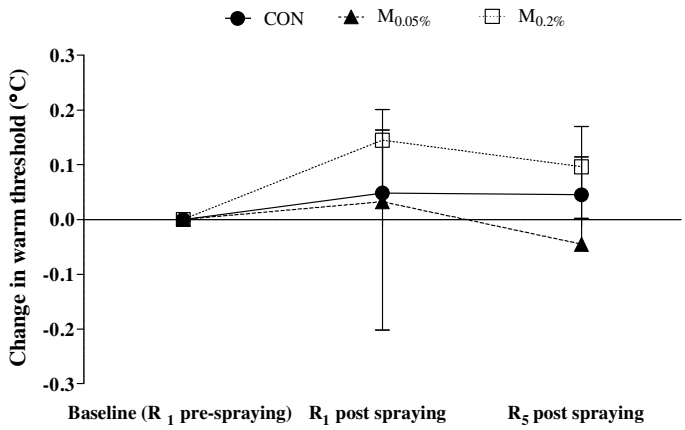


**Figure 46.** Mean (SD) Warm threshold by spray (CON [$n = 6$], $M_{0.05\ \%}$ [$n = 8$], $M_{0.2\ \%}$ [$n = 8$]), measured prior to spraying (baseline), and after spraying in $R_1$ and $R_5$.

## Discussion and Conclusions

This study questioned whether the effects of menthol spraying habituate after repeated exposure to 0.05 % or 0.2 % menthol solution spraying, and secondly, whether repeated 0.2 % menthol exposure caused an habituation in heat storage. Both questions are discussed below.

### *No habituation in heat storage after repeated menthol exposure*

The combination of cycle ergometry and heat stress employed in $Ex_1$ and $Ex_2$ was sufficient to induce a cardiovascular and thermoregulatory challenge. Each group was similar in $\dot{V}O_{2peak}$ and $PO_{peak}$, and all participants maintained a comparable relative work-rate and perceived effort during exercise, as evidenced by the fact that measures of HR, $\dot{V}O_2$ and RPE were comparable across conditions. Dry ambient temperature was also similar across conditions; however, rh was 12 % higher in CON, particularly in $Ex_2$ (equal to an ambient water vapour pressure of 1.4 Kpa) compared to $M_{0.05\ \%}$ and $M_{0.2\ \%}$ (which encountered an ambient water vapour pressure of 1.2 Kpa). The difference in rh can probably be attributed to a fault in the environmental chamber control system, which was observable when the chamber set to 20 °C, 50 % rh. Scheduled maintenance corrected the fault before testing was begun in the $M_{0.05\ \%}$ and $M_{0.2\%}$ conditions. The elevation in relative humidity should not have reduced the capacity for evaporative heat loss in CON compared to the other spray groups because a 12 % difference in rh at an ambient temperature of 20 °C only amounts to a difference in ambient water vapour pressure of 0.2 Kpa (1.4 Kpa [subtract] 1.2 Kpa). Furthermore, this study was primarily concerned with comparing the change in response from the beginning to the end of the week *within* each spray group; so the elevation in rh observed in CON is perhaps of little consequence, particularly as a significant difference was not observed in any of the physiological or perceptual responses in CON. Although $T_{re}$ did appear visually higher in CON $Ex_2$ compared to CON $Ex_1$ there was no difference in the $\Delta T_{re}$ between the two during exercise. Similarly, there was no difference in the $\Delta T_{re}$ observed during exercise in $M_{0.05\ \%}$ or $M_{0.2\ \%}$ from $Ex_1$ to $Ex_2$, nor was there any difference in $\overline{T}_{msk}$, $\overline{T}_b$, finger SkBF, sweat rate, and the respective measures coinciding with the onset of either thermoeffector over this period. Given that 0.2 % menthol has previously been shown to increase the $\Delta T_{re}$ by 0.1 °C compared to Control spraying, a complete habituation should see a similar reduction from $Ex_1$ to $Ex_2$; but the apparent reduction seen in $M_{0.2\ \%}$ over this time (0.03 °C) was in fact smaller than that seen

in CON (0.05 °C); further emphasising that there was no habituation in the 0.2 % menthol-mediated heat storage response.

That 0.2 % menthol spraying did not induce a significant increase in heat storage compared to Control spraying in $Ex_1$ is in contrast to the findings of each previous study in this thesis, and other studies (Kounalakis *et al.,* 2010). When comparing between groups in this study, the influence that menthol exerted over $T_{re}$ was likely clouded with participant differences, whereas previous studies were perhaps more sensitive to the effect of menthol because they served as their own controls. It is also possible that individual differences in exercise-induced metabolic heat production, or environmental factors increased variability.

Metabolic heat production was lower in the resting sessions, which allowed menthol to exert a greater and more measurable influence on thermoregulatory function. During the first half of the 60 minute resting sessions (prior to spraying), an inverse relationship was observed between skin and deep body temperature across all conditions, whereby $T_{re}$ fell and $\overline{T}_{msk}$ rose. Finger skin blood flow was moderately elevated during this pre-spraying period, and this likely encouraged the exchange of thermal energy between the deep body (37 °C) and environment (30 °C); in this way, mean body temperature remained relatively stable. When participants were sprayed at the $30^{th}$ minute of the resting session, mean skin temperature cooled and finger SkBF fell across all conditions, but it appeared to fall more so after 0.2 % menthol spraying. This suggests that the menthol-mediated vasoconstrictor response occurred very quickly after menthol came in contact with the skin. It was not possible to identify the onset of vasodilation in the present study because the 0.2 % menthol-mediated vasoconstrictor response was evident for the remainder of the resting session; hence, under similar conditions, a longer period of observation is required to identify whether menthol shifts the thermoneutral zone whereby warmer temperatures (either skin or ambient air) are required to elicit vasoconstriction *and* vasodilation.

The results of the present study agree with those seen in Study three in that 0.2 % menthol-mediated reduction in skin blood flow has the effect of lowering thermal exchanges between the skin and the environment, thereby encouraging an accumulation of body heat during rest. This response was evident at the beginning of the week in $R_1$, and at the end of the week in $R_5$. This suggests that there is no habituation of the heat storage response after

repeated exposure to 0.2 % menthol, and that 0.05 % menthol spraying does not induce any heat storage response following single or repeated exposures.

***Habituation of thermal sensation after repeated menthol exposure***

Thermal sensations differed significantly between $Ex_1$ and $Ex_2$ and by spray group, and *post-hoc* testing confirmed that participants sprayed with 0.2 % menthol felt significantly cooler than in CON during $Ex_1$, but not during $Ex_2$, suggesting that repeated exposure to 0.2 % menthol results in an habituation of thermal sensation. Specifically, over the testing week, cool sensations diminish by two units on the TS scale, which, by the end of the exercise, equated to a perceptual shift from feeling 'neutral' in $Ex_1$ to 'slightly warm' in $Ex_2$. Although not significant, the 0.05 % menthol group also underwent a shift, whereby over the testing week cool sensations diminished by one TS unit. That 0.05 % menthol spraying did not induce significantly cooler sensations than Control spraying during $Ex_1$ is in contrast to the findings of Study three, but probably can be attributed to increased variability accompanying a between participant study design. As a result, it remains to be clarified whether 0.2 % or 0.05 % menthol still induces cool sensations that are significantly (statistically) cooler than a Control spray, after an habituation has occurred. It is likely that cool sensations would still prevail even after an habituation to 0.05 % menthol spraying, as Study three has shown that thermal sensation was improved by four units on the TS scale during rest, just prior to exercise; hence, losing one TS unit by habituation may still allow for a 3 TS unit improvement. However, this notion requires further clarification.

The finding that thermal sensation underwent an habituation after repeated menthol exposure was partially supported by resting data. Although TS differed significantly between $R_1$ and $R_5$, it did not differ by spray group ($P = 0.08$), and the direction of effect could not be determined through *post-hoc* testing. A number of reasons may explain why TS did not undergo a significant habituation from $R_1$ to $R_5$. Indeed, by the time participants had completed the first resting session ($R_1$, Tuesday morning), they had already undergone one menthol exposure in $Ex_1$ (Monday). This may suggest that the habituation of TS occurred quickly, perhaps after one exposure. Similarly, participants had only undergone five menthol exposures between $R_1$ and $R_5$, but underwent eight exposures from $Ex_1$ to $Ex_2$; hence, there was less of a forcing function between $R_1$ and $R_5$.

Overall, these findings suggest that repeated exposure to menthol results in an habituation of thermal sensation. A thermal adaptation can often be described in terms of behavioural adjustment (which is influenced by our perception) and physiological adaptation (Brager & de Dear, 1998). It should be noted that behavioral adjustment is perhaps the most powerful of these, as our capacity to change an environment is greater; but as participants had no control over their clothing or environment, its influence here was negligible. Likewise, the observation that $T_{re}$ and finger SkBF were altered both before and after repeated menthol exposure suggests that the adaptation was not at the peripheral receptor and perhaps not physiological in nature. Hence, the adaptation was probably located more centrally in higher brain structures, and indicative of a perceptual adaptation, but the underlying mechanisms of this habituation are not clear.

McKemy *et al.,* (2002) and Peier *et al.,* (2002) have shown that a single menthol exposure results in activation of the TRPM8 receptor, which triggers neuronal activations that ascend to higher brain structures, possibly terminating in the somatosensory cortex (perhaps the insular cortex) by way of the thalamus (Craig, 2002). The menthol-mediated perceptual habituation might occur in any of these higher structures. This assertion is not new, and is in fact reminiscent of the conclusions drawn by physiologists studying human adaptation to cold. But unlike cold habituation, the menthol induced habituation of thermal sensation occurs without a change in any physiological variable measured in this study, and although repeated exposures to either cold air (Makinen *et al.,* 2006; Leppaluoto *et al.,* 2001; Bruck *et al.,* 1976) or water (Smolander *et al.,* 2004; Golden & Tipton, 1988) have been shown to cause an habituation in the sensation of coolth and/or thermal discomfort, the underlying mechanisms driving the habituation in either case may not be comparable.

The habituation might also be described in psychological terms. Indeed, psychological skills training prior to cold water immersion has been shown to increase breath hold time by up to 80 % during an initial immersion in cold water (Barwood *et al.,* 2006), and combining this training with repeated cold water immersions can result in a practical improvement in breath hold time above what can be achieved with immersion alone (Barwood *et al.,* 2007). Psychological-based theories in adaptation attribute the perceptual habituation to altered expectations and reduced attentional focus on once novel and unfamiliar stimuli (Veitch & Arkkelin, 1995; Wohlwill, 1975). Perhaps Wohlwill (1975) put it best: ‘‘*The individual cannot afford to respond continually to stimuli or aspects of*

*his milieu of stimulation that are a constant feature of his environment with the intensity of magnitude of affective arousal he exhibits on his initial confrontation with that environment. It is essential that neutralization of affect occur, at least with respect to negatively experienced aspects of the stimulus environment over which the individual cannot exert any control''.*

In any case, the underlying mechanisms driving the menthol-induced habituation of thermal sensation are not clear. There are many areas dedicated to sensory processing within the somatosensory cortex, including the primary and secondary somatosensory corticies, the posterior parietal cortex, and the insular cortex (McGlone & Reilly, 2010). Furthermore, the secondary somatosensory cortex receives input from the primary cortex, and projects to the insular cortex. The insular cortex seems a plausible location for the habituation, as it receives direct stimulation from the spinothalamic tract of lamina I (Craig, 2000) and activates not only in association with subjective feelings, but also attention, cognition, time perception, and subjective expectations to name but a few (Craig, 2009). However, it is important to note that although the role of the insular cortex in perception is undeniably important, the systems neuroscience view holds that cognitive function and perception arise from interactions of brain areas in large scale, distributed networks (Menon & Uddin, 2010). Hence, it is difficult to speculate about the location driving thermal sensory habituation in this study.

There was no measurable habituation in thermal comfort after repeated exposure to either 0.05 % or 0.2 % menthol during the exercise or resting sessions. Further, irritation did not reduce after repeated exposure to menthol. It is important to note however, that not all individuals noted irritation; in fact, only half of those in either menthol spray group noted any irritation. Interestingly, about 60 % of these individuals noted greater irritation at the beginning of the week than at the end of the week. These findings support the notion that there is a large individual difference in the perception of irritation with menthol exposure.

The influence of menthol on a participant's ability to detect warmth (*i.e.* warm threshold) was also investigated. Green (1992) has shown that menthol may suppress sensations of warmth; this raises the possibility that individuals who are exposed to menthol in hot environments may not perceive temperatures that, although are not extreme enough to cause tissue damage, may represent a dangerous thermoregulatory challenge. Although 0.2

% menthol spraying appeared to induce an elevation in the warm threshold compared to baseline measures by 0.1 °C, there was no significant difference between any of the conditions following single or repeated exposure to either menthol spray. This finding is in general agreement with Yosipovitch *et al.,* (1996) and Namer *et al.,* (2005) who applied 10 % (620 mg · 100 $cm^{-2}$) menthol, and 40 % menthol (640 mg · 100 $cm^{-2}$) respectively to the forearm, neither of whom found a difference in the threshold for warm sensation.

Given these findings, the null hypothesis that there will be no habituation of the heat storage response following 0.2 % menthol spraying can not be rejected. The null hypothesis that there will be no habituation of thermal sensation after repeated exposure to menthol can be rejected in favour of the alternative, that after repeated exposure to menthol, thermal sensation undergoes an habituation.

In summary, previous studies have shown that a single exposure to 0.2 % menthol increases heat storage, and both 0.2 % and 0.05 % menthol induces cool sensations, compared to a Control spray. This study has shown there is no habituation of heat storage after repeated exposure to 0.2 % menthol, and confirm that this response is mediated by an increase in vasoconstrictor tone. Alternatively, thermal sensation does undergo an habituation, most significantly after 0.2 % menthol spraying. Given that the menthol-mediated vasoconstrictor response was evident before and after repeated 0.2 % menthol sprayings, the peripheral receptor is not likely to have been the site of the habituation, as its activation is thought to be causal in initiating the cold defence response. This suggests that the habituation in thermal sensation was most probably located more centrally, in higher brain structures, but it is not clear whether it occurred somewhere along the central neuro-anatomical pathway, or perhaps in the somatosensory cortex, possibly in the insular cortex, or whether it occurred in the larger scale distributed networks associated with memory retrieval and expectation. In any case, these findings raise the possibility of using a water-based 0.05 % menthol spray to enhance sensations of coolth with some capacity to enhance evaporative heat loss during rest or exercise in the heat.

# Chapter 8

# General discussion

The work presented in this thesis tested the hypotheses that: a water-based solution containing ethanol and/or menthol could enhance evaporative cooling when sprayed on the skin compared to a Control condition, thereby lowering heat storage and improving thermal perceptions during rest and exercise in warm, humid conditions; and menthol may also improve perceptions independent of temperature by direct stimulation of cold receptors. The hypothesis that menthol-mediated cool sensations would not undergo any habituation after repeated exposures was also tested. The results support the general acceptance of these hypotheses, with the qualification that menthol's influence on thermoregulation and perception differs by dose and fades with time, and the perceptual responses undergo some degree of habituation, while the thermoregulatory responses do not. This section will discuss the finer points of these findings. The first section discusses menthol's influence over thermoregulation, and the second, over perception. The last section discusses the performance implications of using a water-based menthol spray.

## The influence of a water-based menthol spray on body temperature regulation

This section begins by discussing the thermoregulatory implications of wetting the skin with an ethanol and/or menthol-based water spray. The underlying mechanisms driving the menthol-mediated heat storage response are then considered. The section concludes by discussing the stimulus characteristics of menthol (*i.e.* dose, surface area).

### *Skin surface spraying*

Evaporative heat loss from the skin is not determined by sweat rate alone; but rather by a combination of factors, including the permeability of ones' clothing, and the environmental conditions, particularly the ambient water vapour pressure. Evaporative heat loss occurs when sweat, water, or another liquid stores up sufficient thermal energy from the skin to undergo a phase change from liquid to gas; during the conversion heat is removed, and the skin is cooled. Lowering skin temperature widens the thermal gradient between the skin and the blood, which encourages thermal exchanges between the deep body and the environment. But, if the vapour pressure in the air is equal to or higher than at the skin, the pressure gradient is reduced and liquid will not evaporate, so the skin will not cool.

In this thesis, relative humidity remained constant around 70 % (30 °C dry bulb, 26 °C wet bulb), which is equivalent to a water vapour pressure of 2.97 Kilopascals (Kpa). In order to optimise evaporative heat loss in this environment, the vapour pressure at the skin surface must rise above 2.97 Kpa. To promote this, each participant's breathable shirt was saturated with 100 mL of water. This oversaturation, combined with an elevation in mean skin temperature accompanying exercise (which approached 35 °C), resulted in an estimated vapour pressure at the skin of 5.9 Kpa. With this favourable pressure gradient, water was free to evaporate from the skin. As the latent heat for water vaporisation is 2.45 kJ per mL (Godts *et al*., 2005), each 100 mL spray had the potential to remove 245 kJ of thermal energy from the skin. But it should be noted that this level of saturation can also be achieved through sweating alone; in fact, participants in Study three produced a comparable volume of sweat in just the last 10 minutes of exercise. Of course, this rate of sweating was not observed immediately, it took 25 minutes of exercising at $PO_{45\%}$ to attain it. The key point is that evaporative heat loss can be enhanced in humid environments by wetting the skin any time prior to the onset or plateau of sweating. Support for this notion came in Study three, which showed that when spraying took place 15 minutes prior to the onset of exercise, the $\Delta\overline{T}_b$ required to initiate sweating after exercise had begun was actually negative (*i.e.* - 0.1 °C). Given that sweating is normally initiated when $\overline{T}_b$ exceeds the sweating threshold, which is usually 0.2 °C to 0.5 °C above the thermoneutral state (Taylor *et al.,* 2008), these findings demonstrate the evaporative capacity of water when sprayed on the skin, and support the use of water spraying prior to exercise as a means of enhancing evaporative heat loss.

Skin surface spraying can also enhance evaporative heat loss *during* exercise, but this depends upon the prevailing level of sweat production, which is influenced by exercise intensity. For example, Study one showed that water-only spraying lowered both skin and rectal temperature compared to a no-spray condition during low intensity stepping exercise (sweat rate was not measured). This finding is in sharp contrast to Bassett *et al.,* (1987), who employed 120 minutes of treadmill running in conditions (29 °C, 66 % rh) similar to those used in this thesis, and examined the physiological responses to repeated skin wetting (50 mL water spraying every 10 minutes). They found that although water spraying lowered skin temperature compared to a no-spray condition, it did not influence deep body temperature. As the intensity of exercise was greater in the study by Bassett *et al.,* (1987) (mean HR was 155 beats $\cdot$ $min^{-1}$) compared to Study one of this thesis (mean HR was 95

beats · $min^{-1}$), sweat production likely differed; hence, the evaporative potential of the water spray was perhaps greater in Study one compared to the study by Bassett *et al.,* (1987). Wetting the skin of treadmill runners who are already sweating (1 L · $h^{-1}$; Bassett *et al.,* 1987) is perhaps inefficient because any additional water will drip-off before it stores enough thermal energy to evaporate. Incidentally, each bead of dripped water will absorb some thermal energy as it runs off, which perhaps explains why Bassett *et al.,* (1987) observed lower skin temperatures. So, the key point is that water spraying has the potential to enhance evaporative skin cooling when it is used on participants who have a comparably low level of sweat production; or more generally, during lower intensity exercise, or in dry, hot or windy conditions. But this is not to say that additional skin wetting would fail to enhance evaporative heat loss, it only means that some of the water and sweat will drip off the body without evaporating.

Study one showed that wetting the skin with a water-based menthol solution containing 20 % ethanol can enhance evaporative heat loss compared to no spraying and water spraying. This is because one gram of ethanol evaporates after storing only 920 joules of energy; water however, requires 2,450 joules to vaporise (Godts *et al.,* 2005). The ethanol/menthol spray in Study one enhanced skin cooling for approximately 30 minutes post-spraying; after this time there was little difference in skin temperatures between all of the conditions, suggesting that the ethanol had completely evaporated. It seems then that the optimum spraying frequency of an ethanol solution is every 30 minutes in similar conditions. Although it is reasonable to assume that a similar ethanol-based spray will improve evaporative heat loss when used prior to the onset and plateau of sweating as previously described for water spraying, it is not clear whether there is any benefit to spraying after participants have reached a plateau in sweat production; or more specifically, whether the ethanol would vaporise before it drips off. In any case, this raises the possibility of combining 0.05 % menthol (not 0.2 %, as it results in heat storage) together with 20 % ethanol and water to enhance the evaporative cooling potential of the 0.05 % menthol-based water spray. Interestingly, this 0.05 % menthol + 20 % ethanol solution could be reapplied up to four times (separated by 30 minutes) in one exercise session before the quantity of menthol known to induce a heat storage response accumulates on the skin (0.05 % x 4 = 0.2 %, or 2.1 mg · 100 $cm^{-2}$).

***Menthol and the underlying mechanisms that encourage heat storage***

Studies three and four showed that 0.2 % menthol, when sprayed on the skin, caused a reduction in skin blood flow that was greater than that observed with Control spraying. The enhanced vasoconstrictor tone was not mediated by a fall in skin temperature, but rather by activation of the TRPM8 receptor (McKemy *et al.,* 2002; Peier *et al.,* 2002). Menthol-mediated activation of these cold receptors enhanced the proportional neuronal output from the skin, such that higher brain structures received a cold input that would have been interpreted as a fall in skin temperature. As a result, and because individuals were in the thermoneutral zone, hypothalamic structures attempted to stabilise mean body temperature by allowing deep body temperature to drift up. But because the additional vasoconstriction mediated by menthol was independent of, and not due to, a fall in mean skin temperature, mean body temperature rose with rectal temperature.

Given that the regulation of mean body temperature at rest is characterised by an inverse relationship between skin and deep body temperature (Savage & Brengelmann, 1996), it is possible to estimate the reduction in mean skin temperature required to offset the menthol-mediated rise in rectal temperature. For example, if mean body temperature is maintained around 35.1 °C (as it was in Study four, CON $R_1$, end of the resting session), a 0.15 °C menthol-mediated elevation in rectal temperature (equating to 37.1 °C in Study four, $M_{0.2\ \%}$, $R_1$) would need to be offset with a mean skin temperature of 31.48 °C according to Burton's (1935) formula ($[T_{re} \cdot 0.65] + [\overline{T}_{msk} \cdot 0.35]$). However, the actual mean skin temperature value in the 0.2 % menthol spray condition was 0.5 °C warmer than this (32 °C). This suggests that the menthol-mediated increase in neuronal output arising from peripheral cold thermoreceptors was equivalent to a 0.5 °C fall in mean skin temperature, and the body reacted by regulating mean body temperature as previously described.

Studies three and four showed that 0.2 % menthol-mediated activation of cold receptors was associated with an enhanced vasoconstriction and a lower skin blood flow in a warm (~ 30 °C) environment compared to a Control condition. During rest in a thermoneutral environment, mean body temperature is regulated by altering skin blood flow (Savage & Brengelmann, 1996). In this zone, maximal states of vasoconstriction and vasodilation are primarily influenced by neuronal activity arising from thermoreceptors. Of course, thermoreceptor activity is most often influenced by skin temperature, which can be influenced by a number of factors, including ambient temperature (Mekjavic & Eiken,

2006) or water spraying (*i.e.* Savage & Brengelmann, 1996). But the work undertaken in this thesis has shown that the activity arising from thermoreceptors in the thermoneutral zone can also be influenced by menthol. The influence of menthol on the thermoneutral zone is shown in Figure 47.

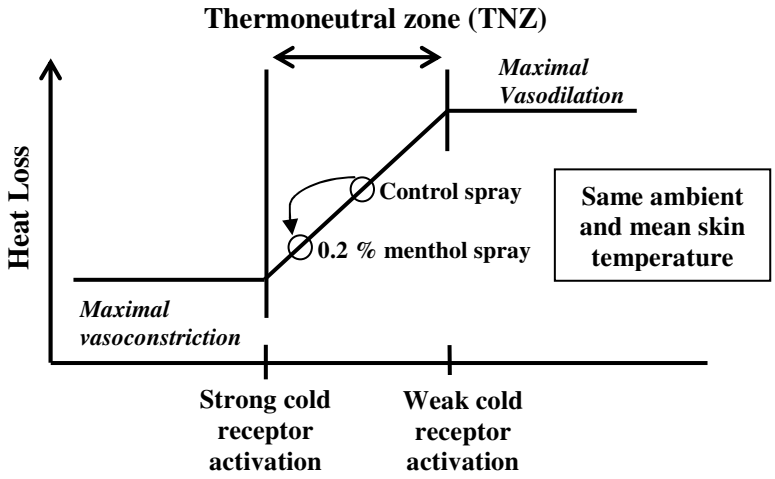


**Figure 47**. The influence of menthol on the thermoneutral zone (TNZ).

Menthol-mediated vasoconstriction, as shown in Figure 47, is independent of skin and ambient temperature. For this reason, labelling its horizontal axis with the skin temperatures often associated with thermoneutrality (*i.e.* 33 °C and 35 °C, Savage & Brengelmann 1996) is perhaps misleading, as is labelling it with ambient temperature. It is perhaps more accurate to describe the thermoneutral zone as being bound by neuronal activity arising from thermoreceptors. Of course, this is an oversimplification for a number of reasons. First, it was not possible to identify the onset of vasodilation at rest in any of the studies presented in this thesis because exercise was undertaken either before its onset (Study three), or the period of observation was too short (Study four), so it is difficult to speculate about any menthol-mediated delay in vasodilation. Furthermore, although vasoconstriction is mediated by an increase in cold receptor input to higher brain structures (Morrison & Nakamura, 2011), it is also mediated by the nitric oxide system which is inhibited during local cooling in hairy regions of the skin (Johnson & Kellog, 2010). Likewise, a combination of central vasoconstrictor withdrawal, as well as peripheral inhibition of vasoconstrictor function accounts for most of the increase in skin blood flow observed during local warming at rest (Johnson, 2010). Although Figure 47 is an over-simplification of the neuronal input driving vasomotion in the thermoneutral zone, its purpose is to focus on the neuronal drive arising from peripheral thermoreceptors as an input to higher thermoregulatory centres, as opposed to skin or ambient temperature.

The influence that menthol exerted on thermoregulation was limited to the thermoneutral zone; but its effect was visible during exercise. For example, Study three showed that both rectal and mean body temperature were visibly elevated by 0.1 °C prior to the onset of exercise in the 0.2 % menthol spray condition (Study three), and this difference persisted throughout 45 minutes of exercise compared to the Control condition. In this way, it appears that menthol encouraged an 'up-regulation' of body temperature at rest, whereby sweating began at a higher absolute rectal and mean body temperature during exercise in the 0.2 % menthol condition compared to the Control condition; but importantly, the change in mean body or rectal temperature that was required to initiate sweating (calculated from the onset of exercise) did not differ between the two conditions. These findings suggest that 0.2 % menthol exerted a non-thermal (chemical) influence over thermoregulation in the thermoneutral zone, but did not appear to influence thermoeffector function (sweating) during exercise. This finding is in contrast to Kounalakis *et al.,* (2010), who reported that menthol raises the $T_{re}$ that is required to initiate sweating *and* delays its onset by minutes. As discussed below, the disparity is perhaps due to the difference in dose used or area stimulated between studies. Kounalakis *et al*., (2010) made reference to the theory of reciprocal cross inhibition (Sherrington, 1906; Bazett, 1949; Bligh, 1998) to explain the observation that exercising participants stimulated by menthol showed a withdrawal of the thermoeffectors for heat dissipation (*i.e.* primarily sweating). Although this theory is used in a variety of physiological systems (*e.g.* the innervation of agonist and antagonist muscle; Sherrington, 1906) and offers an alternative to set-point theory (Mekjavic & Eiken, 2006), it is not clear how the hypothalamus integrates thermal information and triggers thermo-regulatory responses. Given that none of the studies undertaken in this thesis showed a menthol-mediated withdrawal of sudomotor function, it is difficult to confirm or refute the importance of reciprocal cross inhibition in thermoregulatory function at the systems level.

Although 0.2 % menthol spraying represented a sufficient forcing function to perturb thermal homeostasis upon a single exposure (Study three), the heat storage response did not undergo an habituation after repeated exposure (Study four), a finding which is perhaps counter-intuitive to adaptation theory (Tipton *et al.,* 2008). Study three demonstrated that 0.2 % menthol spraying resulted in an elevation in $T_{re}$ that was 0.16 °C above the Control condition, but there was no significant elevation in $\overline{T}_b$; in fact $\overline{T}_b$ showed a plateau nearing the end of exercise, indicating that thermal balance was achieved. This may suggest that

the added heat storage encountered with 0.2 % menthol spraying is perhaps more statistically relevant than practically. As such, it remains to be determined whether a larger dose or % BSA exposed to menthol might increase the forcing function such that an habituation might be observable after repeated exposures. Further research is required to clarify this question.

***The stimulus characteristics of menthol (dose and surface area)***

In the context of the studies undertaken in this thesis, menthol-mediated vasoconstriction seems to be dose-related, with larger doses representing a greater forcing function to perturb thermal balance than smaller ones. This was shown in Studies three and four when 0.2 % menthol spraying resulted in finger vasoconstriction and heat storage at rest, but 0.05 % menthol spraying did not. In the context of the wider literature, the dose shown to influence vasomotion in the studies undertaken in this thesis is the smallest ever reported so to do; specifically, 2.1 mg · 100 $cm^{-2}$ of menthol induced vasoconstriction. In contrast to this, Olive *et al.,* (2010) and Kounalakis *et al.,* (2010) observed vasoconstriction, but with 17.5 mg · 100 $cm^{-2}$ and 27.5 mg · 100 $cm^{-2}$, respectively. Importantly, the work undertaken in this thesis exposed the entire upper body (55 % BSA) of participants to menthol, while Olive *et al.,* (2010) exposed only the forearm (< 3 % BSA), while Kounalakis *et al.,* (2010) exposed the entire body (95 % BSA). Of course, the study by Kounalakis *et al.,* (2010) also observed a withdrawal of sudomotor function during exercise, perhaps supporting the notion of menthol's dose-dependent influence on this effector response. But Kounalakis *et al.,* exposed the whole body to the higher menthol dose, raising the possibility that the percentage of body surface area (% BSA) exposed to menthol may alter the forcing function (spatial summation). A similar effect has been observed with other modalities, whereby removing clothing insulation from the torso and limbs during a cold water immersion increases the skin surface area exposed to the cold water, which increases the ventilatory response during the first few moments of the immersion (Tipton & Golden, 1987). But, the notion that the % BSA exposed to menthol influences stimulus strength is hypothetical at present because no studies have systematically explored this question.

**The influence of a water-based menthol spray on perception**

That menthol enhances sensations of coolth is not new (Anonymous, 1924); but interestingly, its target receptor, TRPM8, has only recently been identified (McKemy *et al.,* 2002; Peier *et al.,* 2002). So it is perhaps not surprising to learn that the central neuro-

anatomical pathways by which menthol exerts its influence over perception are not precisely known. They may follow the same pathways as cold temperature, terminating in the somatosensory cortex (perhaps the insular cortex) by way of the lateral spinothalamic tract and the thalamus (Craig, 2002). The work undertaken in this thesis did not directly measure receptor function or brain activity; but because menthol has been shown to activate the cold receptor TRPM8 (McKemy *et al.,* 2002; Peier *et al.,* 2002) and elicit cool sensations when applied to the skin (Watson *et al.,* 1978; Green, 1992; Yosipovitch *et al.,* 1996; Wasner *et al.,* 2004; Namer *et al.,* 2005; Green & Schoen, 2007), the role that the TRPM8 receptor plays in cold temperature detection and perception in humans can slightly be nuanced by comparing reports of thermal perceptions between a menthol condition and a Control condition.

The published version of Study three (Gillis *et al.,* 2010) is the first reported attempt to apply menthol to a large % BSA during rest or exercise, and then measure perception. Although previous work has assessed the influence of menthol on perception, it was primarily in response to small amounts applied on the forearm (Watson *et al.,* 1978; Green, 1992; Yosipovitch *et al.,* 1996; Wasner *et al.,* 2004; Namer *et al.,* 2005; Green & Schoen, 2007). For that reason, this thesis offers a novel insight into the influence menthol (and the cold receptor TRPM8) exerts over more global perceptions; particularly, thermal sensation, thermal comfort and irritation; each of which are discussed below.

***Thermal sensation***

The findings presented in this thesis are in general agreement with previous literature in that menthol elicits cool sensations; despite large differences in the dose and % BSA exposed. Prior to this thesis, in which 0.52 and 2.1 mg · 100 $cm^{-2}$ of menthol were sprayed over 55 % BSA, the smallest dose of menthol reported to elicit cool sensations (when applied to the forearm) was equivalent to 5 mg · 100 $cm^{-2}$ (Watson *et al.,* 1978). Aside from this, most studies used a much larger dose, ranging from 60 mg · 100 $cm^{-2}$ (Green, 1992) to 3,200 mg · 100 $cm^{-2}$ (Wasner *et al.,* 2004) applied to the forearm.

The influence menthol exerts over thermal sensation is dose-related, but it also exhibits a time-dependent characteristic whereby larger doses are thought to exert their influence longer than smaller ones (Watson *et al.,* 1978). It is not clear whether the decay in thermal sensation over time follows from receptor adaptation, absorption of menthol in the skin and its clearance into the blood (Martin *et al.,* 2004), or whether other factors interact to

quicken its diminishment, such as the elevation in mean body temperature with exercise, or the subsequent increase in RPE. The experimental designs employed in this thesis do not provide a clear answer to this question; as the number and timing of spraying differed across studies, along with the exercise intensity and ambient temperature. But based upon the findings of each study in this thesis, 0.2 % and 0.05 % menthol exert their influence for only short periods of time (15 to 30 minutes). So to optimise the perception of cooling, a spray might be deployed later in exercise when participants feel hotter or perhaps repeatedly during exercise; but repeated application may be associated with greater heat storage because the quantity of menthol on the skin will double with every spraying, and larger doses of menthol represent a greater perturbation to thermal balance.

Menthol also seems to have a dose-related influence on thermal sensation; but Study three showed that increasing the dose four fold, from 0.5 to 2.1 mg · 100 $cm^{-2}$, did not result in a four fold improvement in thermal sensation. This finding supports the assertion originally made by Green (1992), that a doubling of a menthol dose does not coincide with a doubling of perceptual cooling, and suggests that the effect of menthol was nearing saturation at the lower dose. This explains why reducing the dose of menthol from 0.2 % to 0.05 % in Study three resulted in a preservation of the sensations of coolth; importantly, 0.05 % menthol spraying still induced significantly cooler sensations than the Control spray. It is interesting that the same four fold reduction resulted in the elimination of any observable heat storage following 0.05 % menthol spraying. Indeed, this raises the question of whether the central structures associated with temperature perception are more sensitive to the output of the TRPM8 receptor than those central structures mediating thermoregulation. Perhaps an insight into the sensitivity of higher somatosensory structures to TRPM8 can be gained by the Study four finding that repeated exposure to menthol results in an habituation of thermal sensation, but not in heat storage.

While on the topic of habituation, Studies one and two both featured repeated exposures to menthol (separated by 20 to 60 minutes) and showed that thermal sensation improved comparably after each exposure. This suggests that participants did not undergo any short term adaptation to menthol. But Study four showed that repeated daily 0.2 % menthol spraying resulted in an habituation of thermal sensation. Further work is required to specify the time-course of the menthol-mediated habitation of thermal sensation, and whether either menthol spray still induces significant sensations of coolth after an habituation. It is

likely that cool sensations would still prevail even after an habituation to 0.05 % menthol spraying, as Study three showed that thermal sensation was improved by four units on the TS scale during rest, just prior to exercise; hence, losing one thermal sensation unit to an habituation, as was observed in Study four, would still allow for a three unit improvement.

Separate from the descriptive question of *when* the habituation in thermal sensation occurred, is the mechanic question of *how*. Given that the menthol-mediated vasoconstrictor response was evident before and after repeated 0.2 % menthol sprayings, the peripheral receptor is not likely to have been the site of the habituation, as its activation is thought to be causal in initiating the cold defence response (Kounalakis *et al.,* 2010; Studies three and four). This suggests that the habituation in thermal sensation was most probably located more centrally in higher brain structures, but it is not clear whether it occurred somewhere along the central neuro-anatomical pathway, or perhaps in the somatosensory cortex, possibly in the insular cortex. Or whether it occurred in the larger scale distributed networks associated with memory retrieval and expectation.

***Thermal comfort***

0.2 % menthol spraying induces feelings of thermal discomfort during rest in warm conditions (*i.e.* 30 °C). But it is difficult to determine which factors primarily influenced this response because the strongest feelings of discomfort often coincided with a number of responses including: the coolest sensations; the strongest sensations of irritation; vasoconstriction; and, an elevation in rectal temperature. Physiologically, elevations in deep body temperature may reduce comfort (Frank *et al.,* 1999), and perceptually, an increase in menthol-mediated irritation may have prevented an overall improvement in comfort. Similarly, an increase in skin wettedness has been shown to reduce comfort at rest (Nishi & Gagge, 1977), and is an unavoidable consequence of water spraying. Menthol spraying may also have induced sensations that were 'too cold' (*i.e.* negative allesthesia); a warm stimulus is not always considered comfortable, nor is a cold stimulus always uncomfortable (Cabanac, 1972).

During exercise, 0.2 % menthol spraying improved thermal comfort. But it is difficult to attribute this improvement to a single factor. Indeed, rectal temperature increased along with RPE as exercise continued, while irritation and thermal sensation faded with time. It is possible that elevations in both deep body temperature and RPE accompanying exercise may have 'drowned-out' sensations of irritation, allowing for an improvement to thermal

comfort during exercise with menthol spraying. It is also possible that as participants stored body heat, the negative allesthesial response turned positive, and sensations that were once too cool, eventually proved sufficient to improve thermal comfort. During exercise, individuals are also willing to accept a greater level of discomfort compared to resting conditions, at least with regards to the reduction in comfort associated with skin wettedness (Nishi & Gagge, 1977; Fukazawa & Havenith, 2009). Lastly, the influence of menthol on cool sensations and irritation may have simply worn off, allowing for an improvement in thermal comfort. Further work is required to clarify why thermal comfort is not improved after menthol spraying at rest, and improved with exercise. Alternatively, 0.05 % menthol spraying seems to have no significant influence on thermal comfort at any time, but it appeared to improve it slightly above the Control spray, and comparably with 0.2 % menthol spraying during exercise.

***Irritation***

The finding that menthol gives rise to sensations of irritation is not new; indeed, psychophysical studies applying a range of menthol doses to the forearm (620 to 3200 mg · 100 $cm^{-2}$) consistently reported sensations of burning in addition to cool sensations (Green & Schoen 2007; Namer *et al.,* 2005; Wasner *et al.,* 2004; Yosipovitch *et al.,* 1996). This is probably because up to 50 % of primary neurons that respond to cold and menthol also have the noxious heat receptor TRPV1 (McKemy *et al.,* 2002). Therefore, Green (2004) has suggested that some of the neurons that have the TRPM8 receptor may also project in the nociceptive pathway rather than, or along with the cold pathway. In keeping with previous research, most participants taking part in each study of this thesis noted some form of irritation when exposed to menthol. Although irritation was not measured in Study one, some individuals reported sensations of burning following the combined menthol/ethanol spray. All participants noted some form of irritation, primarily burning, prickling or numbness, after 0.2 % menthol spraying in Study two, and everyone noted irritation in response to both 0.2 % and 0.05 % menthol spraying in Study three. But in Study four, only half of those in either menthol spray group noted irritation. Furthermore, in each study, Control spraying (water) often resulted in minor irritation; however, this was perhaps due to the rubbing of wet fabric on the skin.

Large individual differences in the intensity of irritation were also noted, particularly in Study three. Here, the sensations of irritation that some participants experienced

approached ‘very strong’ following 0.2 % menthol spraying, while others remained ‘barely detectable’. This finding was supported in Study four, when only half of those in either menthol spray condition noted any form of irritation. These findings support the notion that there is a large individual difference in the perception of irritation with menthol exposure, but it is not clear what differentiates the ‘high responders’ from the ‘low responders’.

As with thermal sensation and comfort, menthol-induced sensations of irritation seem to be dose-related and fade with time. The first claim is supported by Study three, which showed that 0.2 % menthol spraying induced significantly greater irritation compared to the Control spray and 0.05 % menthol spraying, this finding confirms the notion put forth by Cliff and Green (1994) that reducing the menthol dose, in this case from 0.2 % to 0.05 %, preserves sensations of coolth and reduces the perception of irritation. Regarding the second claim, reports of irritation were most frequent (with the greatest intensity) shortly after menthol was sprayed but fell thereafter; supporting the notion that menthol induced irritation is time-dependent. It was not possible to determine whether the irritation diminished as a result of receptor adaptation, biological menthol clearance to the blood, or as a result of the elevation in body temperature and RPE associated with exercise.

**Performance implications of a water-based menthol spray**

Dr. Gunnar Borg suggested that the single best indicator of an individual’s physical strain during exercise is their own rating of perceived exertion, or RPE (Borg, 1982). Accordingly, RPE exerts considerable guidance over pace (Tucker, 2009). During maximal exercise, RPE is primarily influenced by physiological responses; indeed, Borg constructed his RPE scale to grow linearly with work-load, and thus with heart rate and oxygen consumption (Borg, 1982). But RPE is also influenced by sensory input from other physiological systems within the body (Borg 1982), and during sub-maximal exercise, a number of other factors, both physiological and perceptual, influence RPE and pace. For example, Nybo and Nielsen (2001) showed that RPE is highly associated with increases in deep body temperature and altered cerebral function, with the latter primarily accounting for reductions in work-rate during prolonged exercise in the heat.

But RPE does not always track changes in deep body temperature (Tucker *et al.,* 2006), and it is also influenced by afferent feedback, such as skin temperature. For example, Tucker *et al.,* (2006) noted a reduction in the self-selected power output of individuals in

the first few minutes of exercise undertaken in hot, humid compared to cool conditions. This altered pace was associated with an increase in skin temperature, suggesting that thermoreceptors sensing the hot, humid conditions were responsible for lowering pace (Tucker *et al.,* 2006). In support of this notion, Schlader *et al.,* (2011a) showed that skin temperature, and the associated perceptions of comfort and sensation, are important inputs determining work-rate. On this note, there is a large body of research supporting the assertion that exercise performance can be modified by a range of psychological interventions, ranging from music (Boutcher & Trenske, 1990; Barwood *et al.,* 2009) to psychological skills training (Barwood *et al.,* 2008). Indeed, each of these studies highlight the brain's role in pacing and in the context of this thesis, raise questions as to whether 0.05 % or 0.2 % menthol spraying could similarly improve pacing strategy or performance.

Given what is known about the thermoregulatory and perceptual drivers of pace, and the influence of menthol on human perception and thermoregulation, it is still not clear whether either menthol spray (0.05 % or 0.2 % menthol) might improve or impair pace during exercise in warm, humid conditions. It is important to note that neither 0.05 % nor 0.2 % menthol spraying influenced RPE during moderate fixed-intensity exercise in any of the studies undertaken in this thesis. However, just because menthol spraying did not improve RPE within the context of this thesis, does not exclude the possibility that it may improve it in another context. For example, during maximal exercise in the heat, thermoreceptors located within the skin and deep in the body convey information on the accumulation of thermal energy to higher brain structures and, if mean body temperature rises uncontrollably, the cumulative neuronal input is thought to produce inhibitory signals that lower power output (and thereby metabolic heat production) to protect the organism from heat injury (Nybo, 2010). The work undertaken in this thesis has shown that 0.2 % menthol spraying increases the neuronal output arising from peripheral cold thermoreceptors to a level that is equivalent to a 0.5 °C fall in mean skin temperature. Given this, it seems possible that spraying a similar menthol solution on trained participants who are close to the 'point of failure' may have the effect of maintaining performance by lessening the inhibitory signals that would otherwise reduce power-output. But such an intervention raises important ethical questions about pushing individuals to exhaustion. Similar ethical questions arise when discussing the use of other ergogenic aids in sport; such as amphetamines, which have been implicated in the death of Tom Simpson,

the British road cyclist who infamously died of exhaustion on the slopes of Mont Ventoux during the Tour de France in 1967.

It is not clear whether menthol would influence maximal self-paced exercise performance in a competition setting; without further research however, it is prudent to suggest there will be no change (null hypothesis) in pace following the use of either menthol spray.

# Chapter 9

# Conclusions and Recommendations

The experiments undertaken in this thesis were designed to assess the 'cost-benefit' of menthol use *before* performance testing was undertaken. The work presented in this thesis tested the hypotheses that: a water-based solution containing ethanol and/or menthol could enhance evaporative cooling when sprayed on the skin compared to a Control condition, thereby lowering heat storage and improving thermal perceptions during rest and exercise in warm, humid conditions; and menthol may also improve perceptions independent of temperature by direct stimulation of cold receptors. The hypothesis that menthol-mediated cool sensations would not undergo an habituation after repeated exposures was also tested.

The results supported the general hypothesis that a water-based upper-body spray containing menthol can increase sensations of coolth compared to no spraying or water-only spraying during rest and exercise in warm, humid conditions, but menthol also influences body temperature regulation. The effect that menthol exerts over perception and thermoregulation differs by dose and fades with time. Specifically, 0.2 % menthol spraying encourages heat storage by enhancing vasoconstriction, and there is no habituation in these responses. 0.05 % menthol spraying did not encourage any additional heat storage compared to a Control spray. Menthol also influenced perception, with a 0.2 % menthol spray promoting cooler sensations and greater irritation than 0.05 % menthol and Control spraying. Compared to a Control spray, 0.2 % menthol reduced thermal comfort during rest and improved it during exercise, while 0.05 % menthol did not alter thermal comfort during rest, and may have improved it during exercise. Neither menthol spray influenced perceived exertion during exercise. Menthol-mediated cool sensations lasted 15 to 30 minutes. Both 0.2 % and 0.05 % menthol sprays underwent an habituation compared to the Control spray, with cool sensations diminishing after repeated daily exposures.

It is concluded that a 0.05 % menthol spray, which induces cool sensations *without* a significant heat storage response, could be considered as a perceptual cooling intervention with some capacity to enhance evaporative heat loss when sprayed on the skin during rest and moderate fixed-intensity exercise in the heat. A 0.2 % menthol spray might be deployed later in exercise, but may increase heat storage and irritation. Further testing is required to identify whether menthol spraying improves maximal exercise performance.

# Chapter 10

# Delimitations, Assumptions and Limitations

## Delimitations

Delimitations are those limits that the researcher knowingly imposes upon an experiment. Generally, delimitations relate to matters of experimental design. For example, the scope of this thesis was initially limited by choosing to assess menthol's influence within the domain of human applied environmental physiology; meaning, whole body thermoregulatory and perceptual responses were of primary concern, rather than, say receptor function or higher brain activity, as an experiment in neurophysiology would likely include. Furthermore, menthol's influence was primarily assessed in warm, humid conditions, rather than cool dry conditions, or whilst immersed in water. This was simply because warm, humid environments perturb thermoregulation and perception, and a menthol-based spray offered possible resolution. Furthermore, moderately fit ($\dot{V}O_{2max}$ 45 $mL \cdot kg^{-1} \cdot min^{-1}$, and a $PO_{peak}$ 350 w; cycle ergometry) males (19 to 29 years old) were selectively recruited for each study in this thesis because they represented a cohort that would likely use said cooling solution. Additionally, the majority of these participants were Sport Science students, enrolled in the Department of Sports and Exercise Science, Portsmouth University, between 2008 and 2011 (*i.e.* when and where this thesis was undertaken) and as such represented a homogenous sample of convenience.

To effectively test the hypotheses presented in this thesis, participants were generally exposed to a thermal load that was sufficient to both challenge thermal perceptions (towards feeling hotter) and body temperature regulation (increased temperature), but moderate enough so they could attain a thermoregulatory steady state or maintain that steady state for some time. So, participants were generally asked to exercise at 45 % of their peak power, for at least 30 minutes in warm, humid conditions, whilst wearing shorts and long sleeve breathable shirts. Furthermore, cycling was primarily chosen as the mode of exercise, due to the low level of skill and familiarisation required in performing the task, but also due to the ease with which work intensity could be monitored and controlled. The exception is Study one, where participants were asked to step 12 times per minute. Although less fit individuals may have experienced a larger rise in deep body temperature than individuals who were more fit, the absolute stepping intensity was comparably low, so

it is hoped that any difference in metabolic heat production between individuals were minimised.

Deep body temperature was monitored at the rectum ($T_{re}$) in all experiments. It is acknowledged that rectal probes are slow in responding because the rectum is surrounded by a large mass of abdominal tissue with low thermal conductivity. Additionally, $T_{re}$ is considered less useful in the dynamic conditions of rapid heating; however, $T_{re}$ was monitored because it is widely used and considered a robust site for deep body temperature measurement when probes are inserted 10 cm beyond the rectum (Tipton, 2006), and it is an established measurement which allows for comparison of our $T_{re}$ results with other studies. Study three and four attempted to address this by measuring deep body temperature at the auditory canal ($T_{au}$) as well as $T_{re}$, but the data were not presented due to incomplete or confounded data sets. Despite surrounding the ear with insulation, the auditory temperature probe was probably influenced by the environmental conditions and/or local heat exchange around the ear. Also, the probe often caused participant irritation and had to be removed, or was often displaced during experimentation.

Skin temperature was measured at three sites in Study one and a thermographic camera captured images that estimated $\overline{T}_{msk}$ of the upper body. In this study, the themographic camera provided an accurate estimation of upper body $\overline{T}_{msk}$, and local skin thermistor recordings provided a real time assessment of the spray's influence on skin temperature. Study two however, measured skin temperature using thermistors only, placed at only four sites, and $\overline{T}_{msk}$ was calculated using Ramanathan's equation (1964). Although the four site formula developed by Ramanathan (1964) has been shown to reliably reflect $\overline{T}_{msk}$ during exercise in the heat, primarily due to the increase in uniformity following peripheral vasodilation, these four sites may not have reliably reflected $\overline{T}_{msk}$ under the conditions of Study two. For example, although exercising in warm, humid conditions will result in a more uniform skin temperature, spraying the upper body with water will result in vasoconstriction (lowering skin blood flow) and less uniformity; hence more sites, both sprayed and unsprayed, were likely required to estimate $\overline{T}_{msk}$ under spraying conditions in Study two. As a result of the participants wearing clothing during exercise in warm, humid conditions (which may increase skin temperature), and spraying the participants clothing with water (which may reduce skin temperature), $\overline{T}_{msk}$ may not be uniform and so may require a greater number of sites to reliably calculate it than the commonly used four site

formula recommended by Ramanathan (1964) during exercise in the heat. Indeed, the increased variability in the $\overline{T}_{msk}$ measure was evident in Study two, so in order to reduce this variability, Studies three and four used more skin sites outside of the clothed, sprayed area. To this end, three sites were measured in the area covered by clothing and sprayed, and the remaining five sites were unclothed and unsprayed. Therefore, $\overline{T}_{msk}$ was calculated in Studies three and four using an eight site weighted formula developed by Olesen (1984), who used stepwise regression to identify the eight highest correlated sites used for predicting $\overline{T}_{msk.}$ The eight site formula correlates very highly ($R^2$ = 0.98) with formulas using 14 skin sites in temperatures ranging from 0 °C to 40 °C (Olesen 1984).

**Assumptions**

Assumptions refer to those facts or statements which are taken for granted, or without proof *per se.* In the context of this thesis, assumptions were made about the true nature of human perception. Specifically, as menthol exerts its greatest influence on perception, the precise quantification of perception is central to this thesis. But little is known about the mechanisms that underpin human perception and its measurement, so a number of assumptions were made to quantify it. It has been assumed that the perception of irritation can be described by some power function, while thermal sensations and thermal comfort are described linearly; the reader should note that it is most likely that all human perception is derived from some power-function, and is not linear (Personal comm-unication in October, 2008, with Dr. B Green, Director and Fellow of the John B. Pierce Laboratory, Yale University, USA). These assumptions are most evident in the types of scales that have been chosen to measure irritation, thermal sensation and thermal comfort. The rationale for choosing each scale lies in their established use in each of their respective fields. Simply put, there is sufficient evidence to support the use of a power function-based scale to measure irritation resulting from menthol applied to hairy skin, like the forearm (Green, 1992). But there is not enough evidence to support the use of such a scale to measure the perceptual response of whole body heating or cooling and its influence on the perception of heat and cold, and the resulting comfort, or discomfort; however, there is evidence to support the use of a bi-polar liner scale in such a scenario (Zhang, 2003).

The dose of menthol sprayed per 100 $cm^2$ was assumed to be the same within participants across repeated measures, and between participants; whereby 0.2 % menthol equated to 2.1 mg · 100 $cm^{-2}$, and 0.05 % menthol equated to 0.52 mg · 100 $cm^{-2}$. This calculation was

limited in two important ways, first, it was based upon an average BSA of 1.76 $m^2$, and second, it was based upon the upper bodies of all participants equating to 55 % of total BSA. With these assumptions in mind, reporting the dose of menthol per 100 $cm^2$ was still more informative than simply reporting percentages alone, even with the errors in its estimation. On another note, although the spraying method was standardised as much as possible, participant BSA inevitably differed, so it is likely that larger participants had a smaller dose per 100 $cm^2$ than smaller individuals. But given that any error was systematic within each individual, and each study featured a within-participant repeated measures design, any influence over the findings of this study are systematic and minimal.

**Limitations**

Limitations can be thought of as those limits that are externally imposed upon each of the studies in this thesis; they also relate to matters of experimental design, or measurement techniques available. For example, each experiment in this thesis was undertaken in a laboratory that was not equipped for measuring receptor function or higher brain activity, so it was not possible to measure afferent outflow from peripheral cold receptors.

Regarding limitations in experimental design, the influence of ethanol or menthol-only spraying could not be determined from Study one, as there was no adequate Control in either case; but as the main aim of this study was to assess the combined menthol/ethanol spray, it was unnecessary to add two additional groups to the experimental design, requiring a total of five repeated measures, rather than only three (M/E, CON, WA).

The effector mechanisms were not assessed in Study two because its primary aim was only to assess the perceptual and deep body temperature response of 0.2 % menthol in isolation of ethanol. When 0.2 % menthol-based spraying was observed to influence heat storage, Study three set out to measure the underlying mechanisms driving this response.

The sensation of irritation was not measured in Study one because it was not clear whether spraying would induce any irritation during exercise in warm, humid conditions. When participants anecdotally noted irritation in this study, attempts were made to describe the quality and number of reports of irritation experienced in Study two, and then to quantify the intensity of irritation using the labelled magnitude scale in Studies three and four.

# Chapter 11

# Recommendations for future experimentation

***The influence of 0.2 % menthol spraying on maximal exercise performance***

The work undertaken in this thesis has shown that 0.2 % menthol spraying increases the neuronal output arising from peripheral cold thermoreceptors to a level that is equivalent to a 0.5 °C fall in mean skin temperature. Given this, it seems possible that spraying a similar menthol solution on trained participants who are close to the 'point of failure' may have the effect of maintaining performance by lessening the inhibitory signals that should otherwise reduce power-output. To test this, participants will complete three conditions: no spraying, water spraying and 0.2 % menthol spraying. During each test, they will first undergo fixed-rate cycle until deep body temperature increased by 1 °C (~ 38 °C); at this point they will begin a 40 km time trial. When their deep body temperature rises by an additional 0.5 °C (~ 38.5 °C) they will be sprayed and continue exercising until they reach the ethical cut-off for the experiment (39 °C), or complete the 40 km time trial. If menthol spraying lessens inhibitory signals, one would expect to see a maintenance of pace (or perhaps an improved pace) in the 0.2 % menthol condition after spraying, compared to the other conditions.

***The influence of a 0.05 % menthol + 20 % ethanol solution spray on thermoregulation and perception during fixed-intensity exercise in warm, humid conditions***

Study one showed that 0.2 % menthol + 20 % ethanol spraying enhanced evaporative heat loss from the skin compared to no spraying and water spraying for 30 minutes post-spraying. Furthermore, the menthol/ethanol solution enhanced thermal sensation compared to the other conditions, but also encouraged heat storage. Study three showed that lowering the menthol dose from 0.2 % to 0.05 % decreased the perception of irritation and preserved sensations of coolth without a heat storage response compared to the Control condition. This raises the possibility of combining 0.05 % menthol and 20 % ethanol in a water-based spray to enhance both evaporative heat loss and cool sensations, without incurring any additional heat storage during fixed-intensity exercise in the heat. The spray could be deployed every 30 minutes, up to four times in one exercise test, before the quantity of menthol on the skin reaches the dose associated with heat storage (*i.e.* 4 x 0.05 % = 0.2 %). The protocol could include both moderate and high intensity exercise to clarify whether ethanol spraying improves evaporative heat loss when sweat production is high.

***The role of menthol-mediated activation of the cold receptor TRPM8 in thermal comfort***

The physiological responses that drive thermal comfort are not precisely known. Some authors have emphasised the importance of mean body temperature as the main input (Flouris & Cheung, 2009), others suggest that skin temperature is perhaps the most important input (Schlader *et al.,* 2009); others still have suggested that both deep body and skin temperature contributed equally, and individually, to thermal comfort (Frank *et al.,* 1999). The work undertaken in this thesis suggest that thermal comfort might be driven more by skin input when menthol is used. The importance of the TRPM8 receptor in driving thermal comfort could be investigated if the neuronal output arising from skin surface application of menthol were to be matched against a comparable level of skin cooling. The degree of vasoconstriction could be used as an index of afferent outflow; but, this would require matching the level of vasoconstriction induced by menthol with the comparable reduction in skin blood flow driven by skin surface cooling. By comparing the thermal comfort of individuals at the 'matched' level of afferent outflow (*i.e.* vasoconstriction), one would gain insight into the importance of the TRPM8 receptor in driving thermal comfort.

***The role of menthol-mediated activation of cold receptor TRPM8 in thermoregulatory behaviour***

If the TRPM8 receptor influences thermal comfort at rest, it stands to reason that it may also be an important modulator of our thermoregulatory behaviour. To test this hypothesis, participants should be free to modify their behaviour (*i.e.* controlling the inlet temperature of a water perfused suit) during a passive cooling challenge (water immersion), with and without menthol. By comparing the fall in mean water temperature at which the thermoregulatory behaviour is initiated between conditions, one would perhaps gain insight into the importance of the TRPM8 receptor in driving thermoregulatory behaviour.

The two previous proposals should include healthy able bodied controls, but also might test other populations that have a reduced sensitivity to temperature stimuli. For example, clarifying the functionality of the TRPM8 cold receptor in elderly individuals may improve our understanding of the numerous underlying mechanisms involved in the age-dependent loss of thermal perception (Guergova & Dufour, 2011). In another example, studying the functionality of the TRPM8 cold receptor in spinal cord injured individuals (above the lesion) may improve our understanding of the thermal adaptations that accompany spinal cord injury (Attia & Engel, 1983).

***The influence of passive versus active elevations in deep body temperature on menthol-mediated sensations of coolth***

Each study undertaken in this thesis showed that the menthol-mediate cool sensations fade with time, but it is not clear whether the decay follows from receptor adaptation, absorption of menthol in the skin and its subsequent clearance into the blood (Martin *et al.,* 2004), or whether other factors interact to quicken its diminishment, such as the elevation in mean body temperature with exercise, or the subsequent increase in perceived exertion. The last two possibilities can be tested by passively (water-bath) and actively (exercise) heating individuals with and without skin surface exposure to menthol. This design can clarify the importance of exercise related factors (perceived exertion, muscle activity, metabolic heat production) to the decay in menthol-mediated cool sensations.

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